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THROMBOCYTOPENIA IN ALCOHOLISM

by

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A THESIS
SUBMITTED TO
THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Thrombocytopenia in Alcoholism submitted by Linda P. Brigden in partial fulfilment of the requirements for the degree of Master of Science.

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Date. May 18, 1973

ABSTRACT

Many reports in the literature over the past five years have documented the occurrence of thrombocytopenia in alcoholics but none to date have offered a clear-cut mechanism. Our purpose was to study thrombocytopenia in these patients and to try to correlate its occurrence and severity with other hematologic changes, liver function, nutritional status and alcoholic intake.

The patients studied were ten males who were admitted to the hospital suffering from acute alcohol intoxication.

We found no correlation between the type or duration of alcoholic intake and the platelet count on admission, although only those patients who had been drinking steadily for more than four days had platelet counts in the thrombocytopenic range. There was also no correlation between the patient's nutritional status (as assessed by the number of meals consumed per day) and his admitting platelet count.

A considerable amount has been written concerning the role of folic acid in the pathogenesis of hematologic changes occurring during alcohol ingestion. We however found no correlation between the patient's folic acid levels on admission and the platelet counts nor was there any correlation between the count and any of the other hematologic parameters that were measured (hemoglobin concentration, white cell count, mean

corpuscular volume, or serum iron concentration).

Similarly, there was no correlation between liver function tests (SGOT, LDH, alkaline phosphatase, bilirubin, prothrombin time) and the platelet count.

All patients experienced a rise in their platelet counts following withdrawal from alcohol, from an average of 67,000/cmm on the day of admission to an average of 576,000/cmm ten days later. This was coincident with the appearance of increased numbers of large platelets on the peripheral smear. At the same time, all but one patient had normal megakaryocytes on bone marrow aspiration.

The survival of Cr^{51}-labelled autologous platelets correlated with the platelet count at the time the survival was initiated. Mean survival values for the alcoholics whose platelet survivals were started five to six days following admission did not differ from those of the normals. The alcoholics whose platelet survivals were started within three days of admission to hospital had half-life values of 2.2-3.3 days (mean 2.6 days) as compared with the normal half-life of 3 - 4.2 days (mean 3.8). Their survival curves differed significantly from that of the normals at Days 1, 2, and 3 only. There was no evidence of increased liver or splenic sequestration of platelets in any of the alcoholics.

In vitro studies of the effect of alcohol on platelet aggregation showed a linear depression of aggregation with increasing concentrations of alcohol

The data would seem to indicate that the ingestion of large amounts of alcohol is associated with a decreased platelet lifespan and an inability of the marrow to compensate. The resulting thrombocytopenia is rectified by the withdrawal of alcohol.

ACKNOWLEDGEMENT

The kind assistance and supervision provided by Dr. A.S. Little is gratefully acknowledged. Thanks are also extended to Doctors R.E. Bell and J.R. Hill who allowed us to use their surface scanning and gamma counting equipment and to Miss Sandi Neil for her excellent typing.

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TABLE OF CONTENTS

CHAPTER PAGE

I. INTORUDCTION: A REVIEW OF THE LITERATURE 1

II. MATERIAL AND METHODS 5

Platelet Survival Studies 5

A - Labelling Method 5

B - Standard 8

C - Sampling 8

D - Calculations 9

Surface Scanning 10

Normal Controls 11

Dietary Histories 11

III. RESULTS 12

Influence of the Type and Duration of Alcoholic Intake and Nutritional Status 12

Folic Acid Deficiency and Alcoholism 14

The Platelet Count and Other Hematologic Parameters 21

The Platelet Count and Liver Function Tests 21

Recovery of the Platelet Count 32

Bone Marrow Morphology 40

Platelet Survivals 43

Surface Counting 49

TABLE OF CONTENTS

CHAPTER PAGE

IV CONCLUSIONS 51

V THE EFFECT OF ALCOHOL ON PLATELET AGGREGATION.... 54

Introduction.......... 54

Materials 55

Effect Produced by ADP and Adrenalin in Human Citrated Plasma 57

Quantitation of Platelet Aggregation 65

Methods 67

Results 67

Discussion 68

VI THOUGHTS ON FURTHER STUDY 80

***

BIBLIOGRAPHY 81

LIST OF TABLES

TABLE PAGE

I. The Type and Duration of Alcoholic Intake 16

II. Abundance of Bone Marrow Megakaryocytes 42

***

LIST OF FIGURES

FIGURE PAGE

1. Plot of Platelet Counts on Admission and the Duration of the "Binge" Preceding Admission 13

2. Plot of Meals Eaten per Day During the Drinking "Binge" and the Platelet Count on Admission 15

3. Plot of Platelet Counts vs Folic Acid Levels on Admission 19

4. Plot of Meals Eaten per Day During the Drinking Binge and the Folic Acid Level on Admission 20

5. Plot of Hemoglobin Concentration Against the Platelet Count on Admission 22

6. Plot of White Cell Counts against the Platelet Count on Admission 23

7. Plot of Mean Corpuscular Volume against the Platelet Count on Admission 24

8. Plot of Serum Iron Concentration against the Platelet Count on Admission 25

9. Plot to Show Platelet Counts and Liver Function Tests of Patient C.H. as Compared with those of Patient A.M. 26

10. Plot to Show the SGOT Level against the Platelet Count on Admission 27

11. Plot to Show the LDH Levels against the Platelet Count on Admission 28

12. Plot to Show the Alkaline Phosphatase Level against the Platelet Count on Admission 29

13. Plot to Show Bilirubin Concentration against the Platelet Count on Admission 30

LIST OF FIGURES

FIGURE PAGE

14. Plot to Show the Prothrombin Time (Expressed as Percentage of Control Value) Against the Platelet Count on Admission 31

15. Plot to Show the Increase in Platelet Counts from the Time of Admission 33

16. Mean Increase in Platelet Count Following Admission (± ISD) 34

17. Plot to Show the Relationship Between the Platelet Half-Life (as Determined by Cr^{51} Autologous Platelet Survivals) and the Platelet Count on the Day on which the Survival was Initiated.... 44

18. Plot to Show the Relationship Between Platelet Half-Life and the Time that had Elapsed Between Admission to Hospital and the Start of the Survival 45

19. (a) Cr^{51} Labelled Autologous Platelet Survival Curves of Ten Normal Individuals 46

(b) Mean ± ISD 46

20. Survival of Cr^{51} Labelled Autologous Platelets in Normal Individuals and in Alcoholics Whose Platelet Survivals were Started 5 - 6 Days Following Admission 47

21. (a) Survival Curves of Seven Alcoholic Patients Whose Platelet Survivals were Started within 3 Days of their Admission to Hospital 48

(b) Mean ± ISD 48

22. Cr^{51} Labelled Autologous Platelet Survival Curves of Ten Normal Individuals and Seven Alcoholic Patients whose Platelet Survivals were Started within 3 Days of their Admission to Hospital 50

LIST OF FIGURES

FIGURE PAGE

23. Primary and Secondary Aggregation in Human Citrated Platelet Rich Plasma Produced by Adrenalin 63

24. Quantitation of Platelet Aggregation 66

25. ADP-Induced Aggregation: Slope of the Primary Wave of Aggregation vs Ethanol Concentration 69

26. Adrenalin-Induced Aggregation: Slope of the Primary Wave of Aggregation vs Ethanol Concentration 70

27. ADP-Induced Aggregation: Change in Light Transmission at 2 Minutes vs Ethanol Concentration 71

28. Adrenalin-Induced Aggregation: Change in Light Transmission at 2 Minutes vs Ethanol Concentration 72

29. ADP-Induced Aggregation: Amplitude at the Peak of the Primary Wave of Aggregation vs Ethanol Concentration 73

30. Adrenalin-Induced Aggregation: Amplitude at the Peak of the Primary Wave of Aggregation vs Ethanol Concentration 74

31. ADP-Induced Aggregation: Slope of the Secondary Wave of Aggregation vs Ethanol Concentration 75

32. Adrenalin-Induced Aggregation: Slope of the Secondary Wave of Aggregation vs Ethanol Concentration 76

***

LIST OF PHOTOGRAPHIC PLATES

PLATE		PAGE
I.	Patient E.H. Peripheral Smear	36
II.	Patient K.M. Peripheral Smear	36
III.	Patient A.N. Peripheral Smear	37
IV.	Patient J.R. Peripheral Smear	37
V.	Patient J.L. Peripheral Smear	38
VI.	Patient J.L. Peripheral Smear	38
VII.	Patient J.L. Peripheral Smear	39
VIII.	Patient J.L. Bone Marrow Smear	39
IX.	Platelets Prior to Aggregation	58
X.	Platelets Prior to Aggregation	59
XI.	Platelets Exhibiting Shape Change Prior to Aggregation	60
XII.	Platelets Exhibiting Shape Change Prior to Aggregation	61
XIII.	Centralization of Platelet Granules Prior to Aggregation	64

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CHAPTER I

INTRODUCTION: A REVIEW OF THE LITERATURE

In the past five years several papers have been published concerning thrombocytopenia in the alcoholic patient. None, to date, have offered a clear cut mechanism.

In 1968, Post and Desforges[1] presented a case history of one alcoholic patient who was given infusions of ethanol during her stay in hospital. Using serial platelet counts and Cr^{51} labelling they were able to demonstrate a fall in the platelet count, reaching minimum levels at 6 - 7 hours post infusion and returning to normal at 24 hours. They were unable to show any corresponding increase in hepatic or splenic activity but did note that the platelets "appeared swollen" while at their lowest level. They postulated an alteration in platelet metabolism caused by the alcohol leading to temporary aggregation and sequestration at an unknown site.

In the same year, Lindenbaum and Hargrove[2] documented ten episodes of thrombocytopenia in five chronic alcoholic patients. They found a 14% incidence of thrombocytopenia amongst alcoholic patients presenting to hospital during a two month period. None of the patients showed any evidence of cirrhosis, spenomegaly or folate deficiency. In all cases platelet counts rose to normal within three to seven days following cessation of ingestion of alcohol and this was followed by a thrombocytosis reaching a peak between the tenth and nineteenth day, with subsequent return to normal. The authors postulated that the thrombocytopenia is a direct toxic effect of alcohol.

In a similar series, Post and Desforges[3] studied twenty episodes of thrombocytopenia in eight patients all showing evidence of acute liver disease. They were unable to demonstrate platelet sequestration in the liver or spleen and showed that thrombocytopenia recurred in spite of normal and very high levels of folic acid, and in the absence of megaloblastic changes in the marrow or peripheral blood. They found slightly shortened platelet survivals in two patients and suggested that alcohol induces a transiently toxic environment for platelets.

These results were confirmed by Cowan and Hines[4] who found no correlation between platelet counts and the hematocrits, white cell counts, serum or red cell folate levels or liver function tests. They estimated that 81% of alcoholic patients admitted to the hospital had initial platelet counts below 150,000/cmm, but noted that there was an increase in the level of circulating platelets after admission, irrespective of whether or not the patient was initially thrombocytopenic. They suggested that suppression of circulating platelets may be achieved by several mechanisms including toxic depression of megakaryocytes, transient splenic sequestration of newly formed platelets, and the presence of a "toxin" in the plasma which reduces platelet survival.

Ryback and Desforges[5] fed alcoholics 210 ml of 86 proof ethanol orally to determine short term effects of alcohol. They then allowed them to drink alcohol ad lib for twelve days, while maintaining a normal diet, to determine long term effects. As was previously observed[1,3], there was an abrupt transient drop in platelet counts five hours after ingestion of the alcohol. This did not correlate with blood levels and was postulated to be due to temporary sequestration related to platelet aggregation and changes in serotonin[6] and epinephrine[7] metabolism. A different mechanism was suggested for the thrombocytopenia which developed gradually over a period of days and returned to normal gradually after cessation of ingestion of alcohol. This was thought to be due to peripheral destruction or marrow suppression or a combination of both.

Numerous hematologic defects have been attributed to alcohol ingestion with differing explanations for their pathogenesis. These include a decreased rate of leukocyte mobilization[8], peripheral granulocytopenia with decreased bone marrow granulocyte reserves[9], an alteration in iron metabolism[10,11], vacuolation of bone marrow pronormoblasts[12] and megaloblastic anemia[14-17]. These have been variously attributed to a suppression of hematopoiesis possibly by an effect on folate metabolism[10,18,19], a direct toxic effect of alcohol on the bone marrow[11,20], a decreased absorption of folic acid.[21]

The above reports offer variable and often conflicting explanations for the hematologic changes occurring in the alcoholic patient. Our purpose was to study thrombocytopenia specifically and to try to correlate its occurrence and severity with type and duration of alcohol intake, nutritional status, other hematologic changes, and degree of liver function.

CHAPTER II

MATERIALS AND METHODS

The patients studied were ten males aged 36 to 70 years (mean = 48.9 years) who were admitted to the University of Alberta Hospital between September, 1971 and September, 1972. All showed signs of acute alcohol intoxication. They received no alcohol at all following admission and were maintained on an average hospital diet. Other medications varied according to the individual need. None of the patients took medications known to interfere with platelet function. Informed consents were obtained from patients before platelet survival studies and bone marrow aspirations were performed.

Routine blood cell counts were done on admission and at various times thereafter using the Coulter Model S counter*. Platelets were counted on the Coulter Model B counter*. Serum folate determinations were done using the isotopic method of Herbert[23] and serum levels of Vitamin B_{12} were measured using the radioimmunoassay method of Tibbling[24]. Serum iron determinations were performed according to the automated method of Zak and Epstein[25,26]. Blood chemistry tests were performed in the routine biochemistry lab on a Technicon SMA-12/60**.

---

* Coulter Electronics Inc. Hialeah, Florida

** Technicon Instruments Corp. Tarrytown, New York

PLATELET SURVIVAL STUDIES:

A. - LABELLING METHOD

Survival of autologous platelets was measured according to the method of Abrahamsen[27]. Whole blood was collected by gravity into vinyl chloride plastic bags[*] containing an ACD (Acid Citrate Dextrose) solution that buffers plasma at ph 6.5 (0.085 M trisodium citrate, 0.065 M citric acid and 2% dextrose)[28-31]. A fresh solution of ACD was made up on the morning of the test and introduced into the bag through a Millipore sterilized filter unit[**]. A 1:6 ratio of anticoagulant to blood was maintained regardless of the volume of blood collected. When the platelet count was greater than 100,000/cmm., 120 ml of blood was collected. For platelet counts below that, 500 ml of blood was withdrawn.

Whole blood was centrifuged at 170G (800 rpm) for 13 mins. at room temperature[*,31]. The platelet rich plasma (PRP) was then transferred to a transfer pack and centrifuged at 400 G (1250 rpm) for 5 mins. to remove any contaminating erythrocytes and leukocytes. The PRP was removed to another transfer pack and centrifuged at 1400 G (2300 rpm) for 15 mins. The supernatant platelet poor plasma (PPP) thus obtained was decanted into another transfer pack and the two bags were left attached, separated only by a hemostat. The platelet button was resuspended in 10 ml of Ringer-citrate-dextrose solution (Ringer's solution, 7 parts; 3.12% trisodium citrate dihydrate, 2 parts; 5% dextrose, 1 part) and incubated with 300 μCi of sterile $Na_2Cr^{51}O_4$[*, 32,33] at room temperature[34-37] for 35-45 mins. The mixture was agitated gently throughout incubation[38].

---

[*] Obtained from Fenwal Laboratories, Division of Baxter Laboratories, Malton, Ontario.

[**] Obtained from Millipore Ltd., Montreal, Quebec. Type GS, 13mm diameter filters with a pore size of 0.22 mm were used throughout.

[*] International Refrigerated Centrifuge - Model PR-6. International Equipment Co., Needham Hts., Mass.

At the completion of labelling time, 10 ml of plasma was removed from the bag containing the PPP and the remainder was allowed to run back into the platelet bag for a final washing. After centrifugation at 1400 G for 15 mins. the supernatant plasma was poured off as completely as possible and the platelet button resuspended in the 10 ml of reserved PPP. 100 mgm of 5% ascorbic acid was added to reduce excess chromate and prevent binding to the red cells of the recipient[39]. Approximately 1 ml of the platelet suspension was retained as a standard and the remainder was weighed and reinfused into the patient.

---

[*] Obtained from Charles E. Frosst and Co. Pointe Claire, Que. at a concentration of 1000 μCi/cc at a specific activity of > 120 mc/mg Cr. The radioactive chromium was never used beyond one half life so that a volume of ≯ 1 ml was added to the platelet suspension.

B. - STANDARD

The standard was divided into two 0.5 ml aliquots and each was placed in a siliconized[*] gamma counting vial[**] and washed with 5 ml of isotonic saline. The mixture was centrifuged at 1400 G (2300 rpm) for 15 mins. and the supernatant separated from the platelet button.

C. - SAMPLING

In the initial studies 9 ml of blood was withdrawn from the patients and mixed with 1 ml of 3% EDTA in saline in a siliconized gamma counting vial. The tube was centrifuged at 400 G (1250 rpm) for 5 mins., the PRP was removed to another vial, and the remaining cells washed with 5 ml of isotonic saline and centrifuged again at 400 G for 5 mins. The washings were then combined with the PPP and centrifuged at 1400 G for 15 mins. The supernatant was then separated from the platelet button.

It was noticed, however, that not only were "yields" uniformly low, even amongst the normals ($\bar{x}$ = 27.6%) but large percentages of the total radioactivity were remaining in the red cell fraction of the samples ($\bar{x}$ = 49.6%). None of the normals had had obvious red cell contamination during incubation and it was therefore concluded that the method of

[*] Siliclad - Clay Adams, Parsippary, N.J.

[**] Amersham/Searle Corporation, Don Mills, Ont., Catalogue # 003324

harvesting platelets from the samples was inadequate. The counting vials measured 11 cm x 1.5 cm and at maximum capacity held only 12.5 ml. It was felt that more efficient centrifugation and washing could be achieved with a smaller volume of blood. A further study was therefore performed with duplicate 4.5 ml samples being withdrawn and added to separate vials containing 0.5 ml 3% EDTA in saline.

A marked difference was noted with 94.9 ± 9.6% (mean ± 1 standard deviation) of the radioactivity residing in the platelet button and only 2.9 ± 2.3% in the red cell fraction. Duplicate platelet samples varied by an average of 3.0 ± 5.0%. On the basis of these results no inferences were drawn from the calculated percent yield and platelet turnover values could not be ascertained. Samples were withdrawn at 30 mins., 1 hr. and 2 hours following infusion of the platelets and daily thereafter.

D. - CALCULATIONS

Blood volumes were taken from a table of predicted normal values[40]. Samples and standards were counted in a gamma deep well counter* for 5 minutes at the end of the study in order to correct for physical decay of Cr^{51} from the time of infusion.

---

* Nuclear Chicago, Des Plaines, Ill.

The radioactivity of the sample platelet button, representing the platelets of either 9 or 4.5 ml of blood was divided by the appropriate number to obtain platelet radioactivity per milliliter and then multiplied by the blood volume to give total circulating platelet radioactivity.

$$\%\ \text{Yield} = \frac{\text{sample count/ml whole blood X blood volume (ml) X 100}}{\text{standard count/ml X weight of infusion}}$$

The yield of platelets at 2 hrs. post infusion was arbitrarily expressed as 100% Survival[41]. Yields on subsequent days were related to this figure and a survival curve was plotted on arithmetic graph paper[39]. The half life was expressed as the day on which the survival curve crossed the 50% intercept. The day of labelling was Day 0, and subsequent days were numbered accordingly.

SURFACE SCANNING:

Surface scanning of body organs was done with a probe counter* having an open-faced 3" crystal attached to a pulse-height analyser and scaler. Two minute counts were taken over the liver, spleen and heart each day and then the individual counts were divided by the value for the heart at Day 0 to compensate for decay. In order that one study might be compared with another - spleen: heart, and liver: heart ratios were calculated.

---

* Nuclear Chicago, Des Plaines, Ill.

NORMAL CONTROLS:

Normal control values were obtained from hematologically normal, healthy young men who participated as volunteer subjects.

DIETARY HISTORIES:

Patients were classified according to dietary habits on the basis of the history obtained at the time of admission and a further dietary history taken at a time when the patient was felt to be more lucid. A classification similar to that set up by Eicher and Hillman was used:[19]

3 meals/day : "Normal diet". 3 meals/day including at least 2 portions of meat and fresh or frozen vegetables.
1-2 meals/day : Diet limited to 1 or 2 meals/day most often consisting of soup and sandwiches.
< 1 meal/day : Virtually no caloric intake other than alcohol.

Patients were also evaluated as to the type and amount of alcohol they had consumed, although in most instances this was difficult to quantitate.

As well as the ten subjects who were studied directly, the charts of six patients who had been admitted to the hospital for acute alcohol intoxication in the previous year were reviewed and their lab values and dietary drinking habits were noted.

CHAPTER III

RESULTS

INFLUENCE OF THE TYPE AND DURATION OF ALCOHOLIC INTAKE AND NUTRITIONAL STATUS :

All patients studied gave a history of chronic alcoholism over the last two to twenty years. All were admitted following "binges" which had lasted anywhere from three days to one month. There was no direct correlation between the duration of the binge and the level of the platelet count (Fig I). However, the patient who had been drinking for less than four days was the only one having a normal platelet count. The fact that this patient had been drinking amounts and types of alcohol comparable to the other patients is suggestive of the fact that a certain time lag is required before the platelet count falls. Post and Desforges [1,3] demonstrated that a 2000 ml intravenous infusion of 5% Ethanol resulted in a fall in the platelet count which began four to six hours after the start of the infusion and reached a minimum value of 10 - 40% of the pre-infusion level at five to seven hours. Within twenty-four hours the platelet count had returned to normal.

Ryback and Desforges [5] confirmed these results but noted a second separate effect as well. Studying three patients who were allowed to consume

PLOT OF PLATELET COUNTS ON ADMISSION AND THE DURATION OF THE "BINGE" PRECEDING ADMISSION

Only those patients who had been drinking up until the time they entered the hospital & who had platelet counts taken immediately following their admission have been included

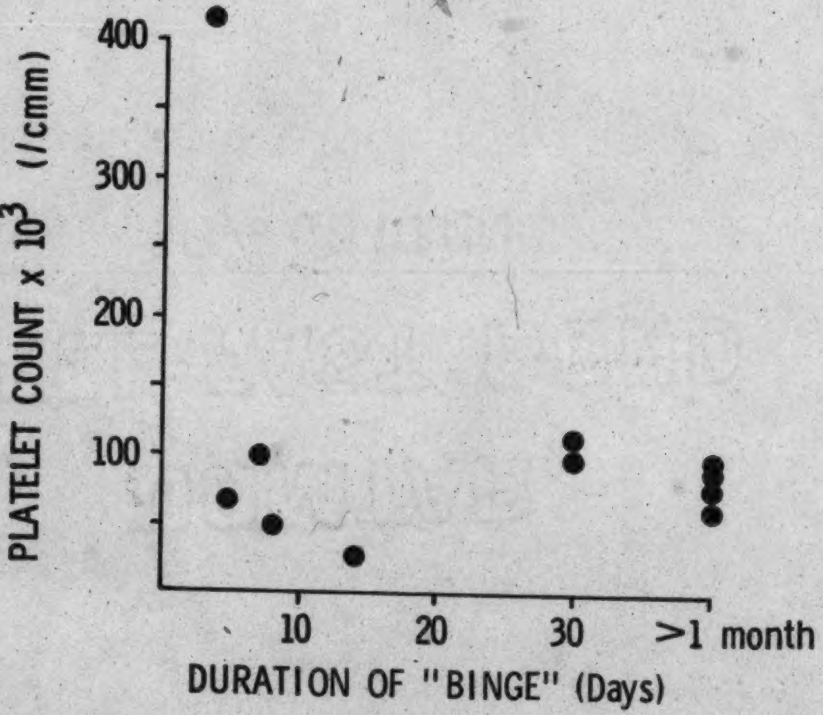


Fig. 1

alcohol ad lib over a period of twelve days, they demonstrated a lowering of the platelet count five hours after the beginning of the experiment. The following day the counts returned to control values. In two of the three patients a second decline was evident by the fourth day and at the end of the week all three were thrombocytopenic. In a similar study Cowan and Hines[4] demonstrated a decline in platelet levels after 8 days of alcohol ingestion, reaching the thrombocytopenic range after 15 days. It would appear that two separate mechanisms are involved and that in the alcoholic patient a severe sustained thrombocytopenia usually occurs only after four to five days of continued drinking.

All patients gave a history of steady drinking all day for varied periods of time. Most consumed the equivalent of at least 26 oz. of whiskey per day and, with exception of one, all ate poorly (< three meals per day) during their binge.

There was no relationship between the type of alcohol consumed and the platelet count (Table I) nor was there any correlation between the number of meals per day and the admitting platelet count (Fig. 2). (Wilcoxon two sample test $p > 0.05$)[42].

FOLIC ACID DEFICIENCY AND ALCOHOLISM :

The occurrence of folate deficiency in the alcoholic patient has been widely recognized. However, the exact relationship between this deficiency

PLOT OF MEALS EATEN PER DAY DURING THE DRINKING "BINGE" AND THE PLATELET COUNT ON ADMISSION

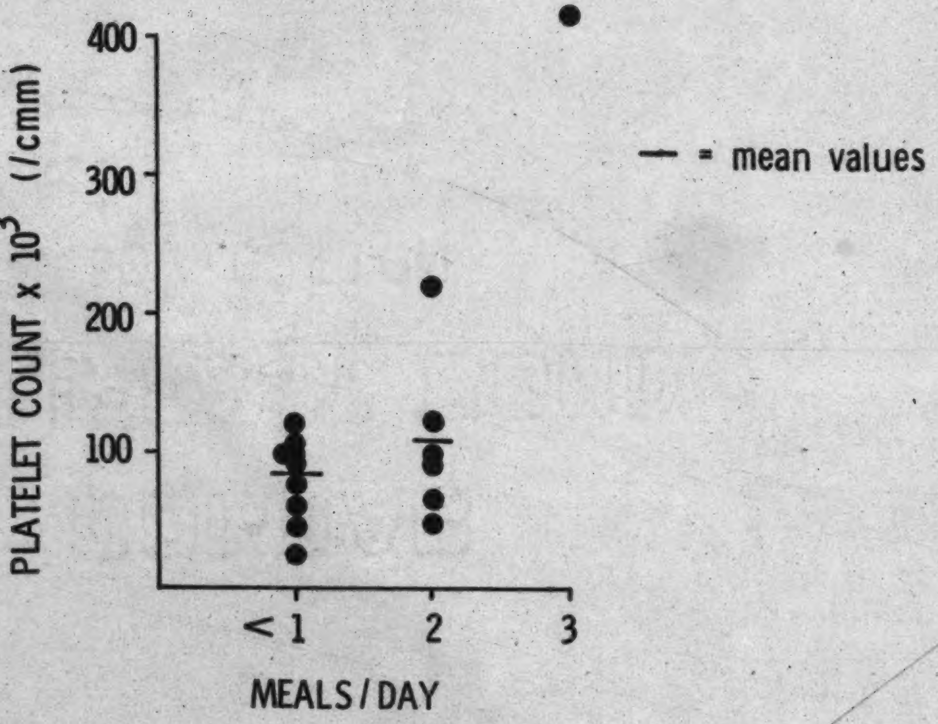


Fig. 2

TABLE I: - THE TYPE AND DURATION OF ALCOHOLIC INTAKE

Patient	Platelet Count on Admission /cmm	ALCOHOLIC INTAKE		
		Steady vs. Binge	Type and Amount	Duration
W.C.	415,000	Binge	beer-4-5 pints/day, wine, whiskey-all day	3-4 days
H.P.	25,000	Binge (nothing for 4 mos. prior)	beer-50 glasses/day, wine-3-4 bottles/day whiskey-2 bottles/day	2 weeks
Z.C.	67,000	Binge	beer, wine, whiskey, gin- all day	5 days
J.L.	47,000	Chronic x 30 yrs Binge	wine, whiskey - all day	8 days
A.M.	47,000	Chronic	whiskey - 8 oz/day	2-3 years
G.P.	100,000	Binge	whiskey - all day	7 days
H.M.	119,000	Binge (stopped 4 days ago)	whiskey - 26 oz/day	10 days
A.M.	122,000	Binge (once a yr for 15 yrs) (stopped 2-3 days ago)	whiskey - 26 oz/day	2-3 weeks
J.R.	98,000	Binge	5 x 26 oz wine/day, some whiskey	3 months
C.H.	90,000	Chronic x 20 yrs Binge	beer-6-12 pints/day, wine-2-3- bottles/day whiskey-2-3- bottles/day	4-5 months
K.M.	106,000	Chronic x 8 yrs	4-6 beer/day	1 month
E.H.	97,000	Chronic x 14 yrs Binge	wine, beer, vanilla, rubbing alcohol - all day	1 month
H.P.	62,000	Binge	beer, 26 oz whiskey, 1 qt. wine/day	2 months
J.D.	77,000	Binge	beer, wine, whiskey, - all day	2 months

and the other hematologic changes that occur as a result of alcoholism has been the subject of some debate. Two major theses seem to exist. One is that alcohol has a direct toxic effect on haemopoiesis[3,11,18,20,43]. The other suggests that there is inhibition or impaired utilization of folic acid in alcoholic patients[10,15,17,19,44]. To begin with, most alcoholics are folate deficient due to inadequate nutrition[13,45]. Not only do they diminish their regular food intake while drinking but, the alcoholic beverages themselves offer very little nutriative value[43,46]. Halsted et al have recently presented evidence that alcohol ingestion over a period of time consistent with the usual binge is instrumental in inhibiting the absorption of folic acid[21], and Cherrick et al have presented evidence that cirrhotic livers have a decreased affinity for folate and presumably, therefore, a decreased capacity for storage[47].

Sullivan and Herbert, the major proponents of the idea that alcohol may cause impaired utilization of folic acid, have shown that alcohol, in amounts readily consumed by "heavy drinkers", suppresses the hematopoietic response of anemic, folate-deficient patients to doses of folic acid in the range of the minimum daily adult folate requirements (i.e. 50 μg)[10]. This suppression could be overcome either with larger doses of folic acid or by cessation of alcohol. Similarly, they found that alcohol may attenuate the response to physiologic amounts (1 μg) of Vitamin B_{12}[10,14] and documented a "rebound thrombocytosis" (similar to that seen in alcoholics taken off alcohol) in non-alcoholic patients with pernicious anemia treated with 5 μgm B_{12} IM/day[13]. They found that this "platelet overshoot" may occur even if the platelet count was normal prior to B_{12} or folate therapy[17,48].

Strong evidence also exists, however, for the occurence of hematologic changes related to alcohol in the absence of nutritional deficiency, suggesting a direct toxic effect of the alcohol. Lindenbaum and Lieber demonstrated vacuolation of marrow pronormoblasts, changes in serum iron and, in four out of nine patients, a depression of the platelet count in well-nourished alcoholics. This fall in the platelet count did not occur until 20 - 33 days after ethanol was started, in contrast to the patient described by Cowan and Hines[4] who was kept on a low folate diet (< 20 μg/day) and became thrombocytopenic after 15 days on ethanol. He, however, received at least 150 ml more whiskey/day than did Lindenbaum and Lieber's patients[12]. Ryback and Desforges demonstrated a fall in the platelet count in three patients within four days to one week after the patients started taking alcohol ad lib[5]. These patients were eating a normal hospital diet which assumedly contained adequate folic acid.

We found no correlation between the patients' folic acid level and platelet count on admission (Fig. 3) ($r = 0.205$ $p. > 0.05$)[49], nor was there any significant difference between the folic acid level of those patients who consumed two meals per day and those who limited their caloric intake almost exclusively to alcohol (Fig. 4) (Wilcoxon two sample test $p > 0.05$)[42].

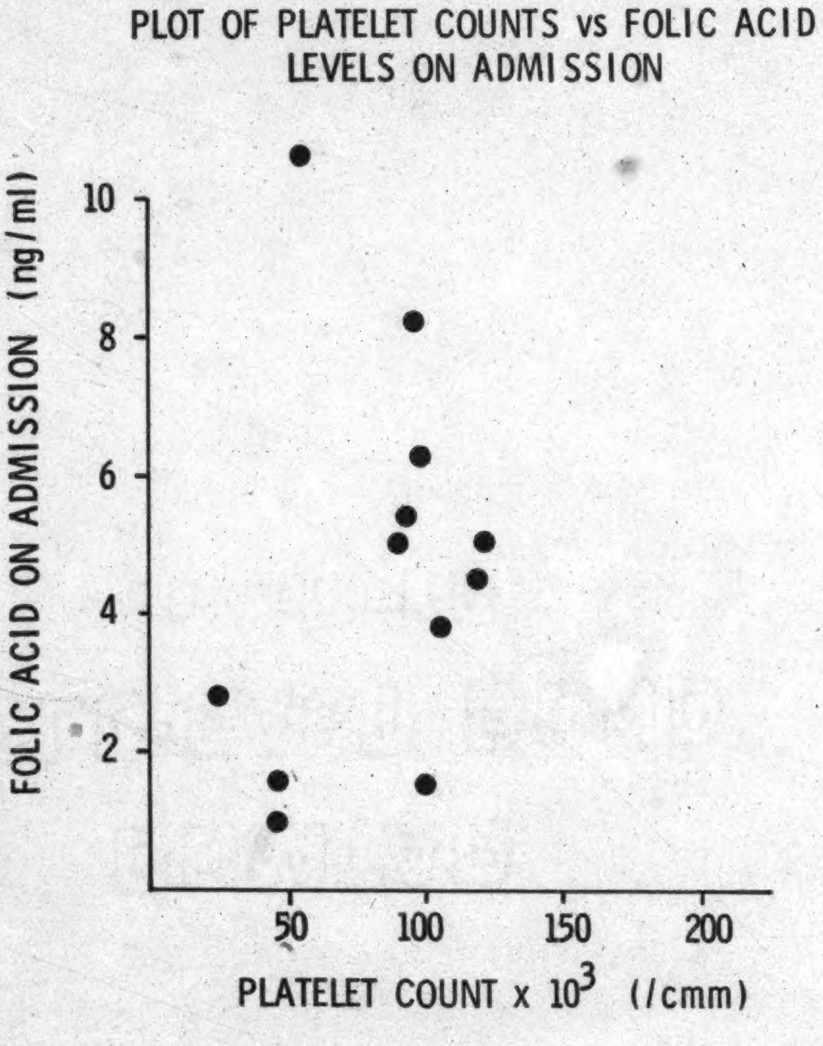

PLOT OF PLATELET COUNTS vs FOLIC ACID LEVELS ON ADMISSION
FOLIC ACID ON ADMISSION (ng/ml)
10
8
6
4
2
50
100
150
200
PLATELET COUNT x 10^3 (/cmm)

Fig. 3

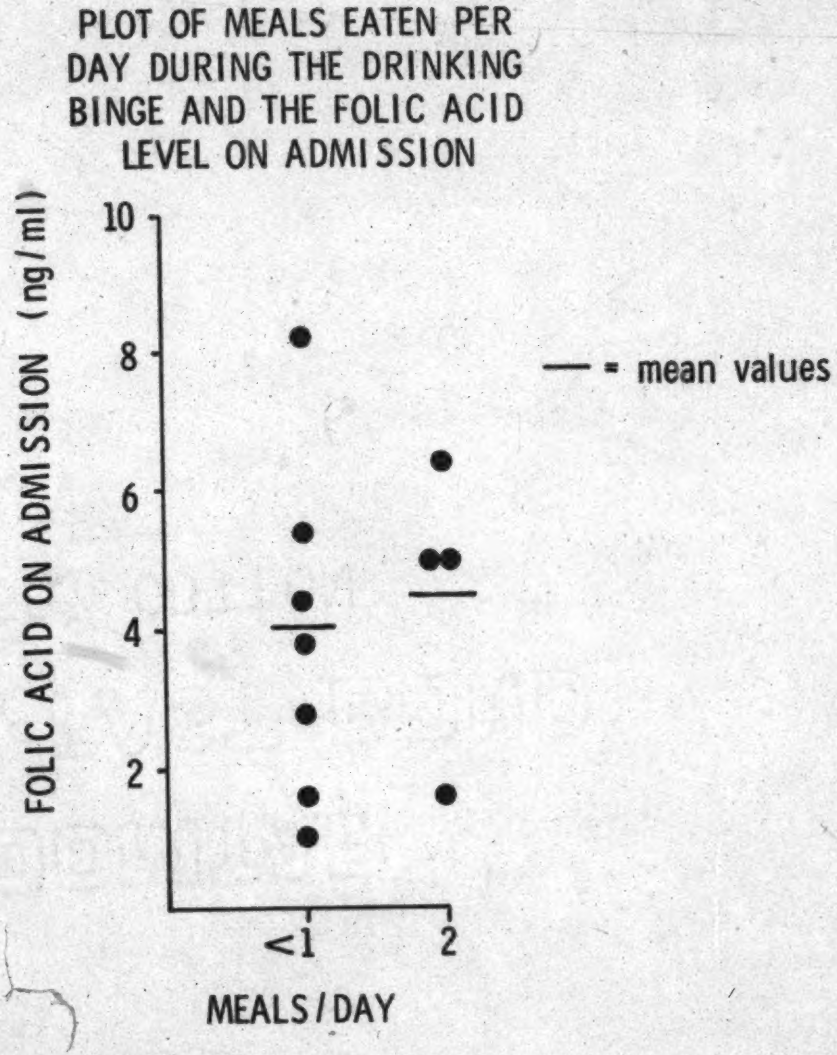

PLOT OF MEALS EATEN PER DAY DURING THE DRINKING BINGE AND THE FOLIC ACID LEVEL ON ADMISSION
FOLIC ACID ON ADMISSION (ng/ml)
10
8
6
4
2
<1
2
MEALS/DAY
— = mean values

Fig. 4

THE PLATELET COUNT AND OTHER HEMATOLOGIC PARAMETERS :

When compared with other hematologic parameters, there was also no correlation between the platelet count and: (i) the hemoglobin concentration (Fig. 5) ($r = 0.02$ $p > 0.1$), (ii) the white cell count (Fig. 6) ($r = 0.31$ $p > 0.1$), (iii) the mean corpuscular volume (Fig. 7) ($r = 0.53$ $p > 0.1$), or (iv) the serum iron concentration (Fig. 8) ($r = -0.18$ $p > 0.1$)[49].

THE PLATELET COUNT AND LIVER FUNCTION TESTS:

It was noted that one patient, C.H., did not respond with the usual rebound thrombocytosis following alcohol withdrawal. Instead, this patient's platelet count returned to normal levels very gradually over a period of 28 days. At the same time his liver function tests (SGOT, LDH, Alk. Phos., and bilirubin concentration) remained abnormal for that period of time. Fig. 9 shows his results as compared with the usual response to alcohol withdrawal as seen in patient A.M. It was thought that perhaps the platelet count was in some way related to the patient's degree of liver damage. In a larger series of patients, however, there was found to be no correlation (when assessed by linear regression) between the platelet count and: - (i) the SGOT level (Fig. 10) ($r = -0.35$ $p > 0.1$), (ii) the LDH level (Fig. 11) ($r = 0.36$ $p > 0.1$), (iii) the alkaline phosphatase

PLOT OF HEMOGLOBIN CONCENTRATION AGAINST THE PLATELET COUNT ON ADMISSION

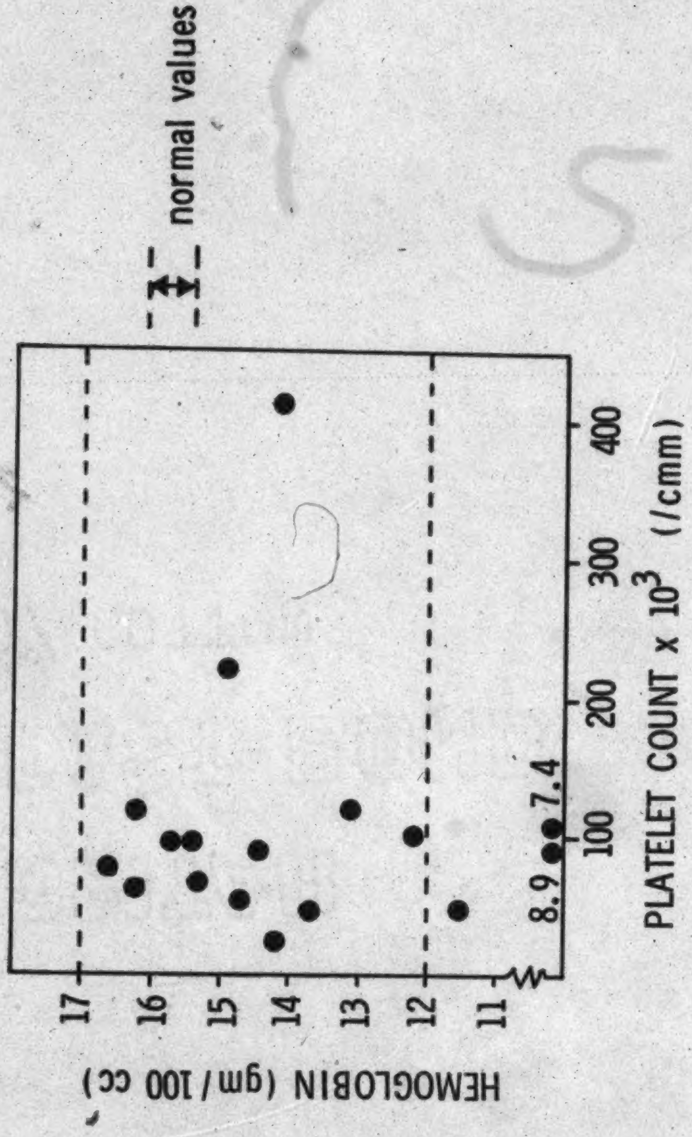
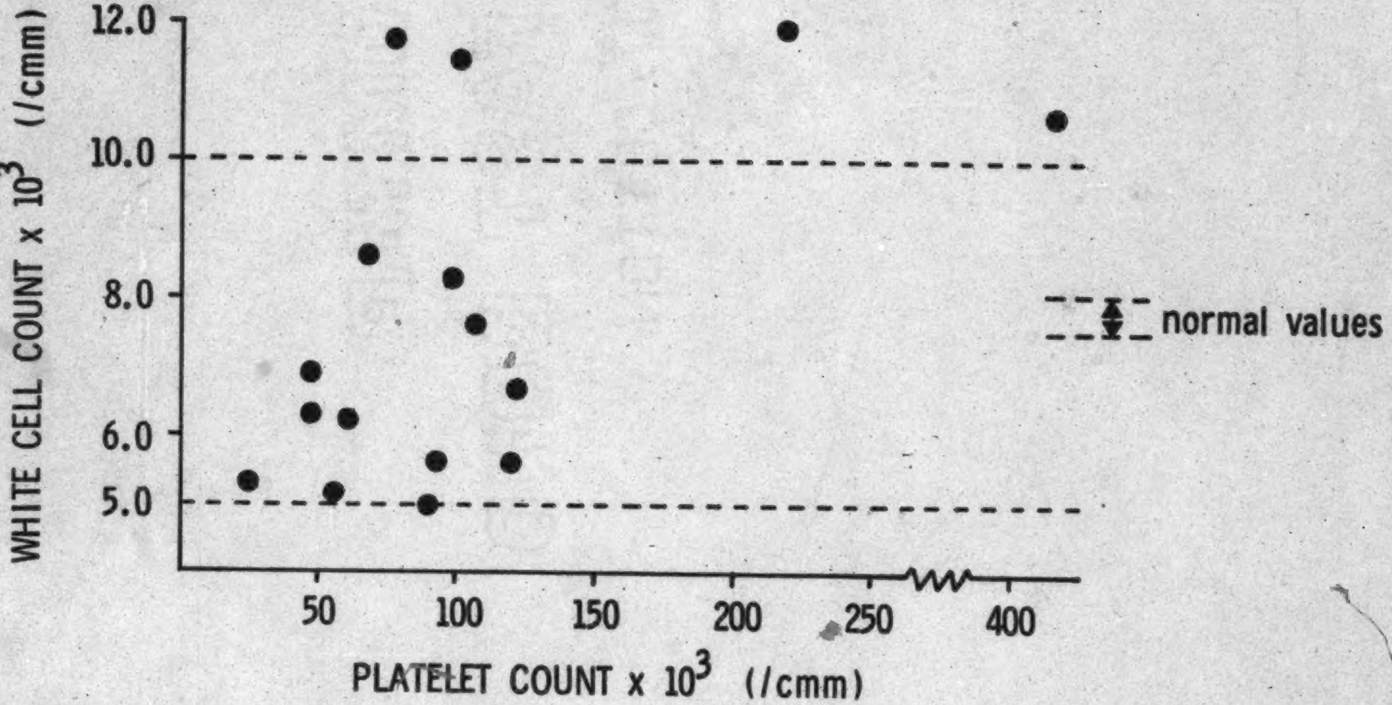


Fig. 5



PLOT OF WHITE CELL COUNTS AGAINST THE PLATELET COUNT ON ADMISSION

Fig. 6

PLOT OF MEAN CORPUSCULAR VOLUME AGAINST THE PLATELET COUNT ON ADMISSION

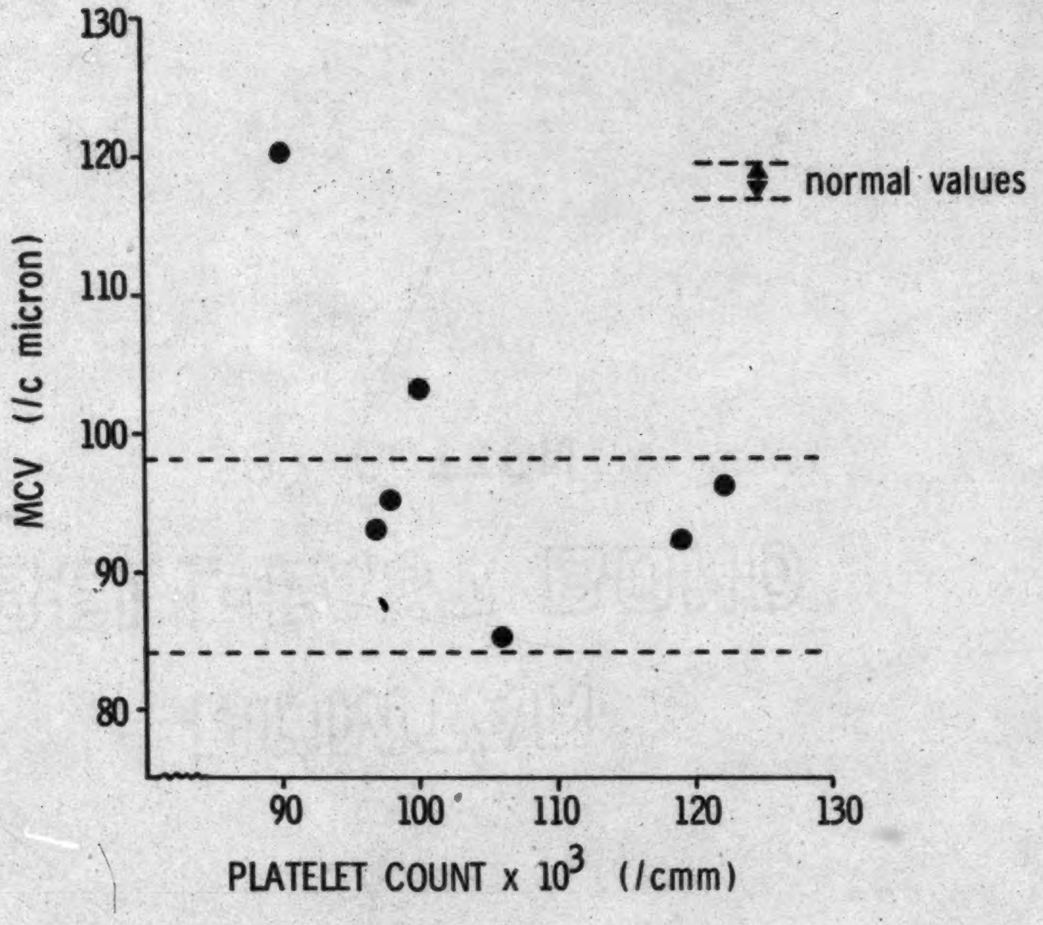


Fig. 7

PLOT OF SERUM IRON CONCENTRATION AGAINST THE PLATELET COUNT ON ADMISSION

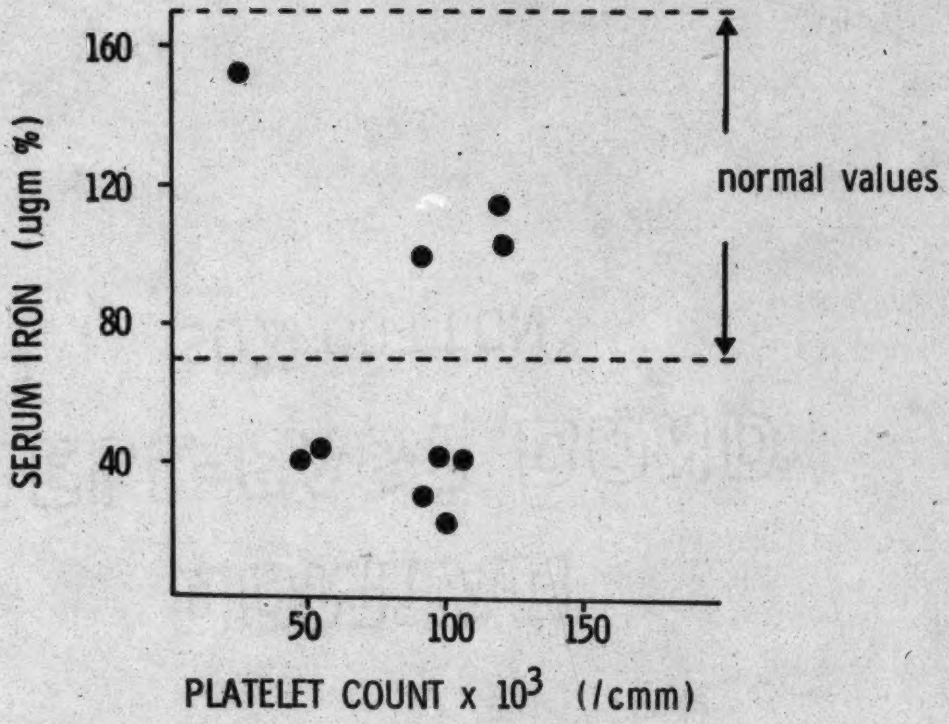


Fig. 8

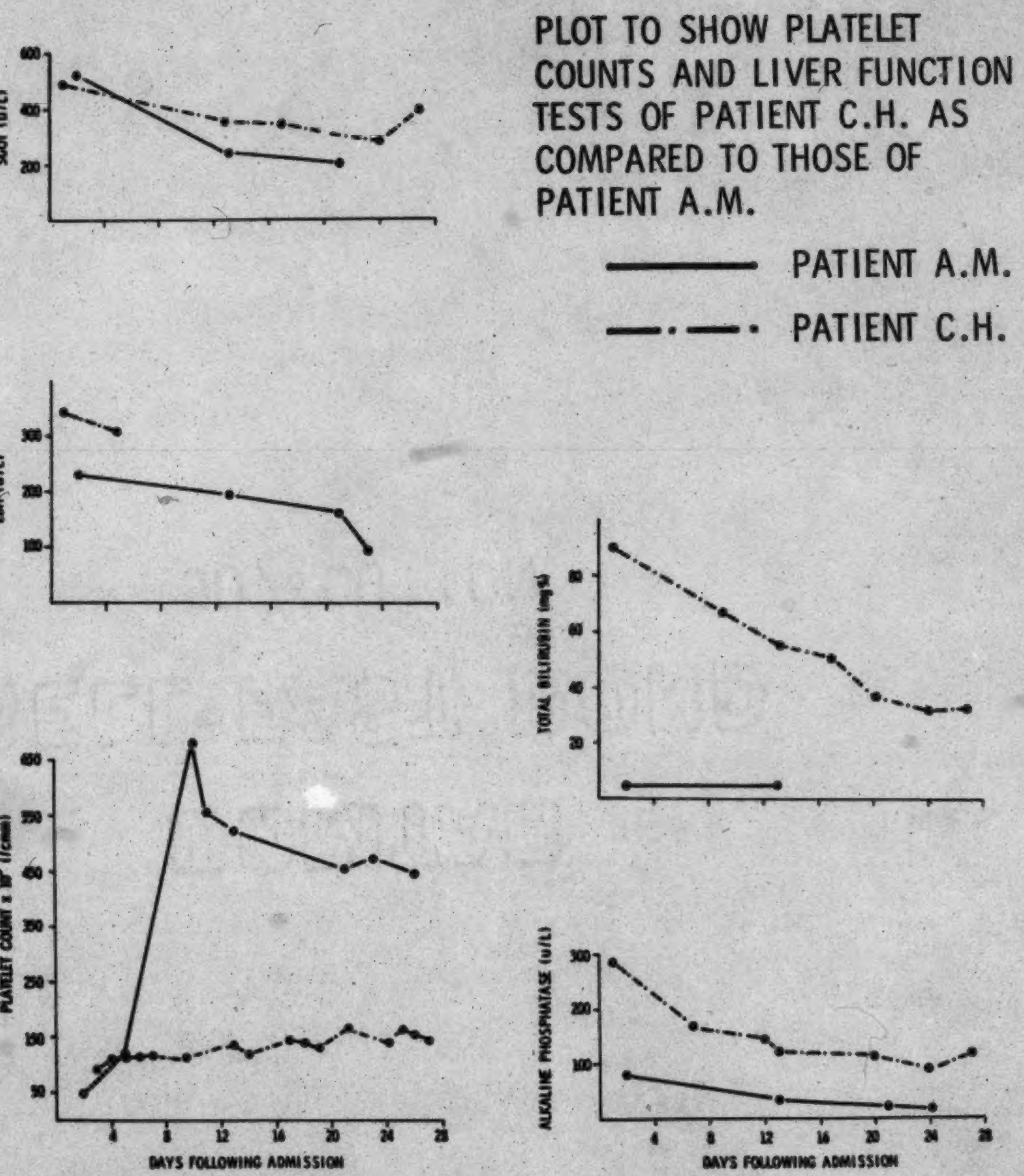

PLOT TO SHOW PLATELET COUNTS AND LIVER FUNCTION TESTS OF PATIENT C.H. AS COMPARED TO THOSE OF PATIENT A.M.
PATIENT A.M.
PATIENT C.H.
SGOT (u/L)
600
400
200
LDH (u/L)
300
200
100
TOTAL BILIRUBIN (mg%)
80
60
40
20
PLATELET COUNT x 10^3 (/cmm)
650
550
450
350
250
150
50
ALKALINE PHOSPHATASE (u/L)
300
200
100
4
8
12
16
20
24
28
DAYS FOLLOWING ADMISSION
DAYS FOLLOWING ADMISSION

Fig. 9

PLOT TO SHOW THE SGOT LEVEL AGAINST THE PLATELET COUNT ON ADMISSION

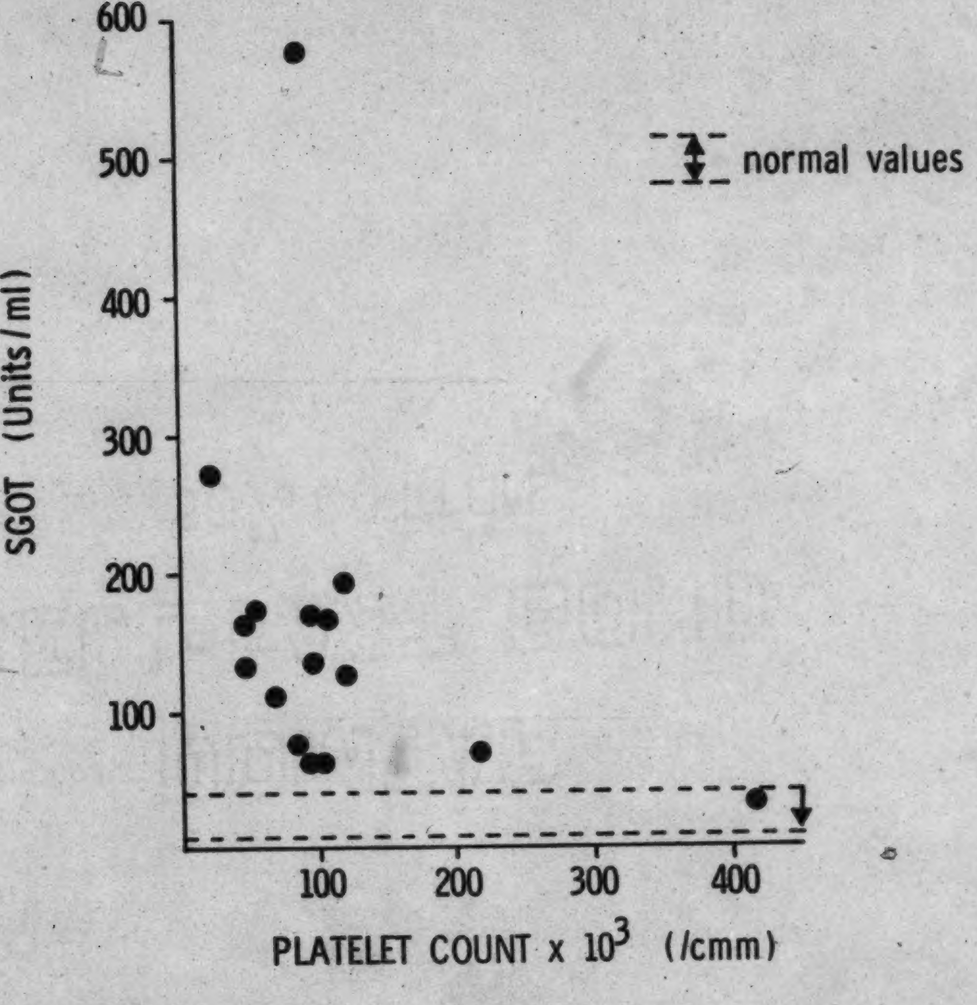


Fig. 10

PLOT TO SHOW LDH LEVELS AGAINST THE PLATELET COUNT ON ADMISSION

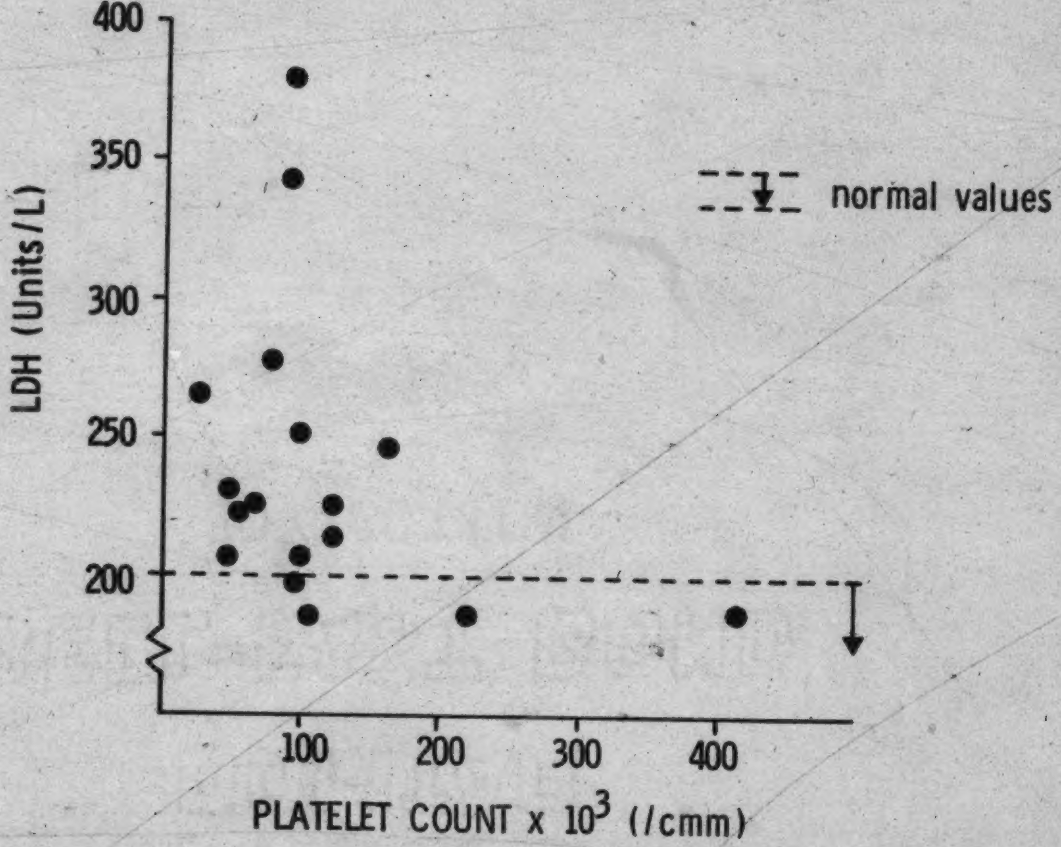


Fig. 11

PLOT TO SHOW THE ALKALINE PHOSPHATASE LEVEL AGAINST THE PLATELET COUNT ON ADMISSION

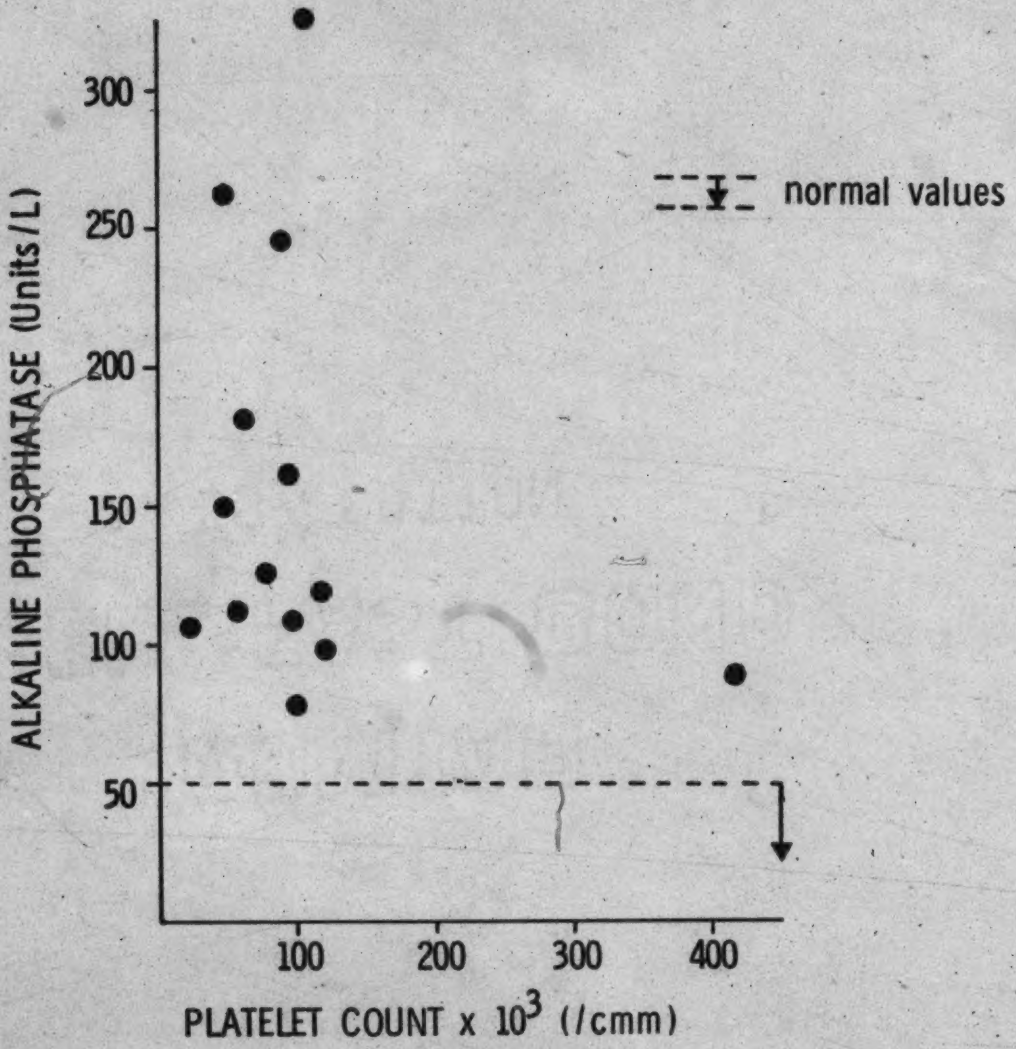


Fig. 12

PLOT TO SHOW BILIRUBIN CONCENTRATION AGAINST THE PLATELET COUNT ON ADMISSION

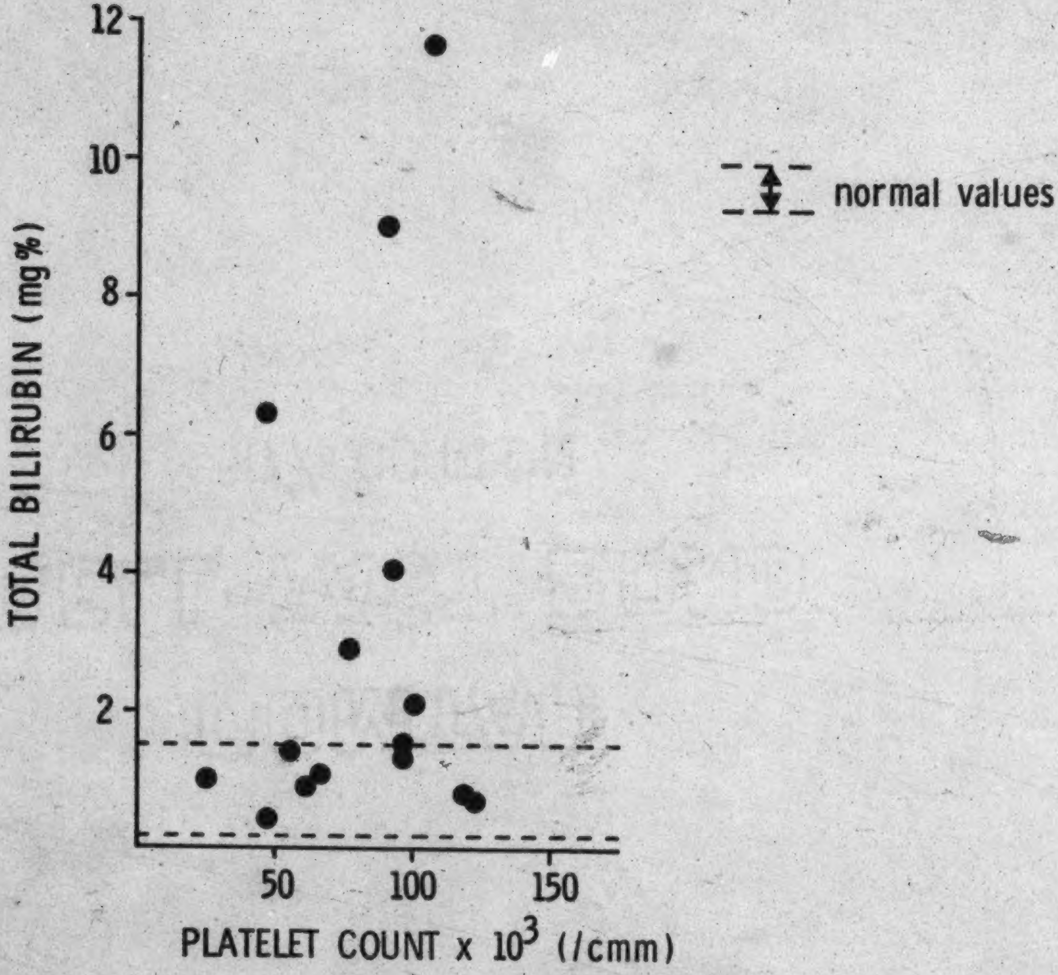


Fig. 13

PLOT TO SHOW THE PROTHROMBIN TIME (EXPRESSED AS PERCENTAGE OF CONTROL VALUE) AGAINST THE PLATELET COUNT ON ADMISSION

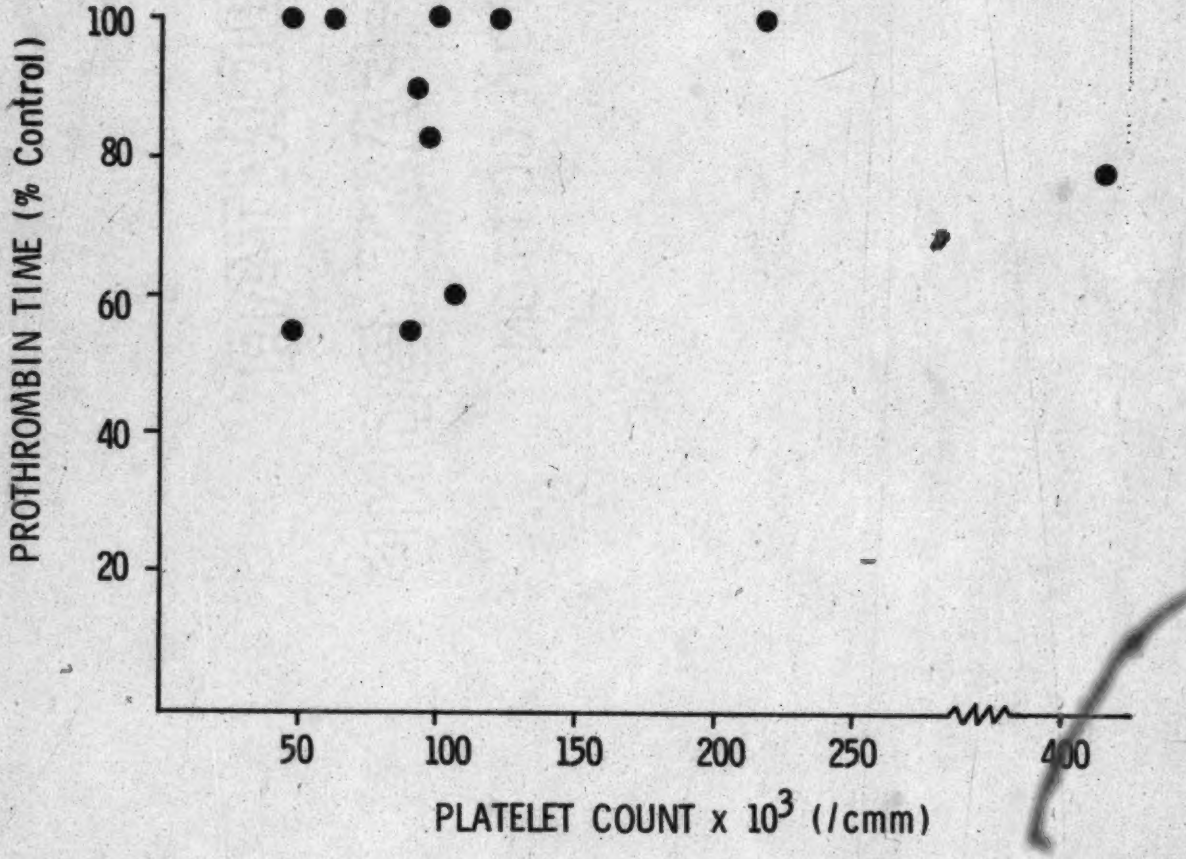


Fig. 14

level (Fig. 12) ($r = -0.26$ $p > 0.1$), (iv) the bilirubin concentration (Fig. 13) ($r = 0.18$ $p > 0.1$), or (v.) the prothrombin time (when expressed as percentage of the control value) (Fig. 14) ($r - 0.03$ $p > 0.1$)[49].

Unfortunately, no platelet survival study was performed on patient C.H. and recovery of his platelet count has not been included with other alcoholics (see below) as it was not felt to be representative.

There is some variation in the number of points on Figs. 5 - 14. Not all patients had each one of the tests performed.

RECOVERY OF THE PLATELET COUNT :

All patients experienced a rise in their platelet counts following withdrawal from alcohol (Fig. 15) from an average of 67,000/cmm on the day of admission to an average of 576,000/cmm ten days later (Fig. 16). Regardless of their initial platelet count, all patients had returned to normal platelet levels within 2 - 7 days following admission. These results are similar to those of Cowan and Hines[4] who found marked increases in platelet counts irrespective of the initial count with peak values occurring five to eighteen days (average 10.7 days) after admission.

Sahud, in a study of platelet size in alcoholic thrombocytopenia[50] demonstrated an increase of three to four times in the platelet size observed on the eighth day as opposed to that seen the day after admission. Larger platelets are generally recognized as being younger more physiologically

PLOT TO SHOW THE INCREASE IN PLATELET COUNTS FROM THE TIME OF ADMISSION

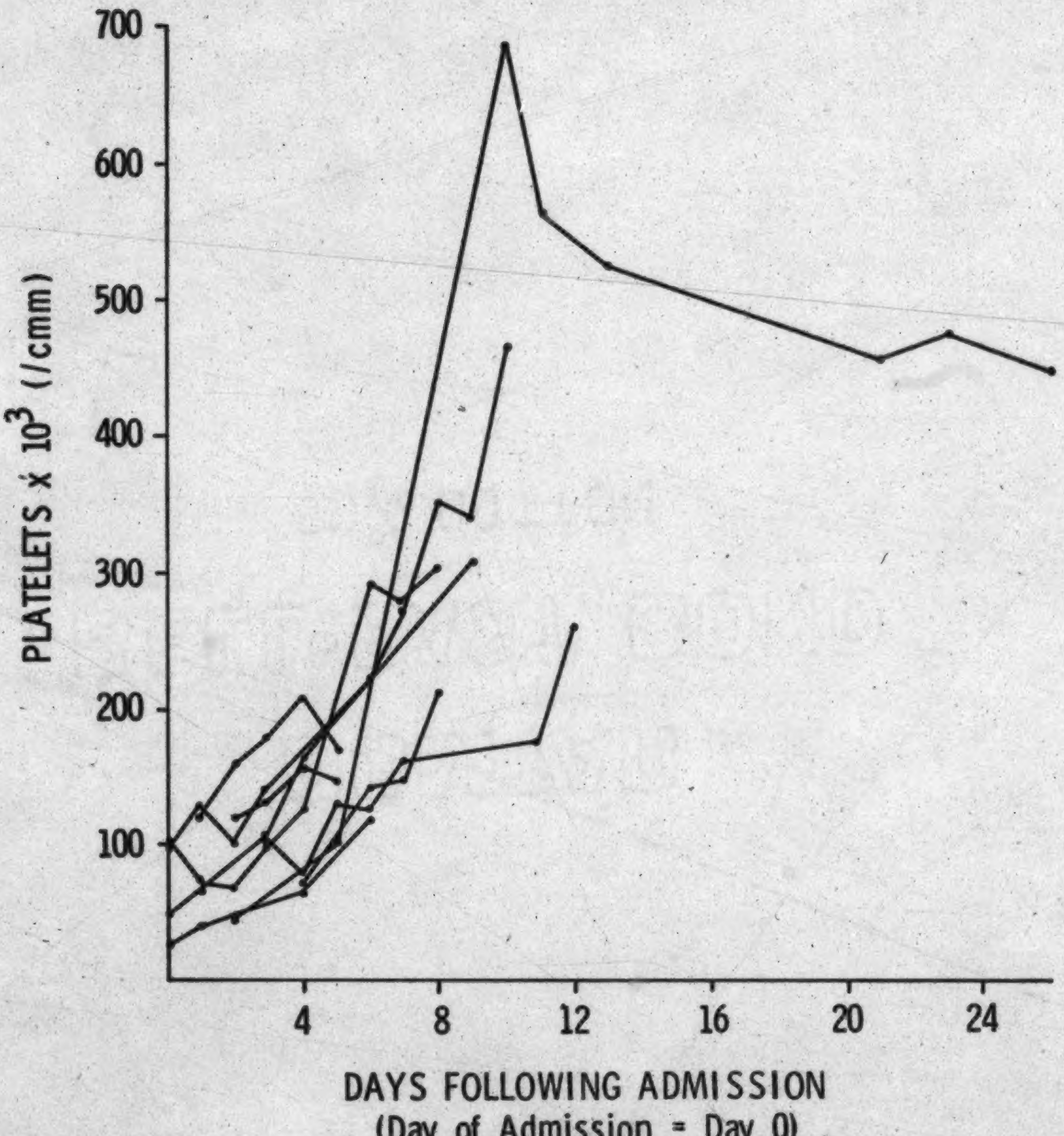
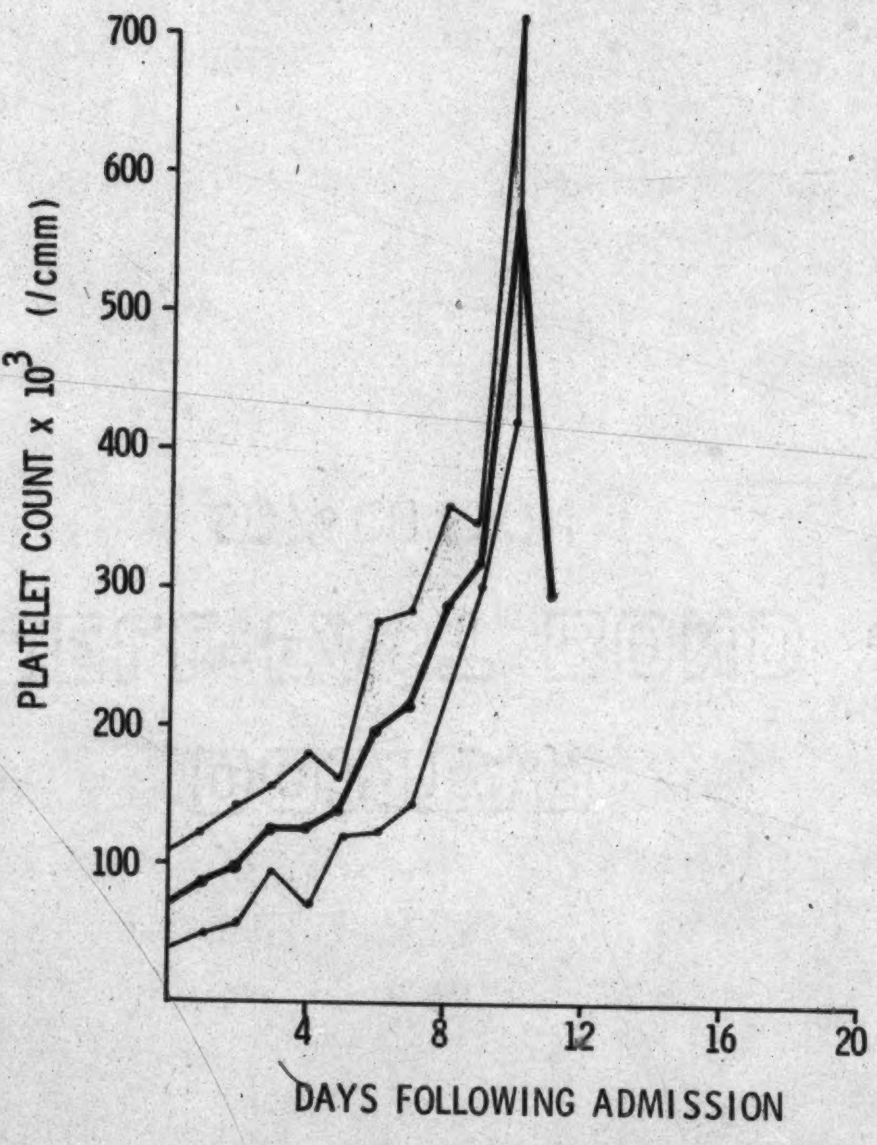


Fig. 15



MEAN INCREASE IN PLATELET COUNT FOLLOWING ADMISSION (± I SD)

Fig. 16

active platelets[51-54] and Karpatkin has demonstrated a reciprocal relationship between the daily recovery of the platelet count in patients recovering from thrombocytopenia and the percentage of large platelets[55]. He suggested that large platelets on the peripheral smear can be employed as a clinical tool for the evaluation of thrombopoiesis.

We therefore examined the peripheral smears of our patients which had been taken within 3 - 6 days after their admission to hospital, at a time when they were recovering from their thrombocytopenia and showing marked increases in their platelet counts. The patients characteristically had increased numbers of large platelets on their peripheral smears. (Plates I - to IV). One patient, J. L., whose platelet count had risen from 67,000/cmm to 164,000/cmm in the previous 3 days, had numerous clumps of platelets throughout his peripheral smear (Plates V, VI and VII) and bone marrow (Plate VIII).

The increase in peripheral platelet count and concurrent appearance of large platelets in the peripheral smear were taken as evidence of an increased rate of effective thrombopoiesis following the withdrawal of alcohol.

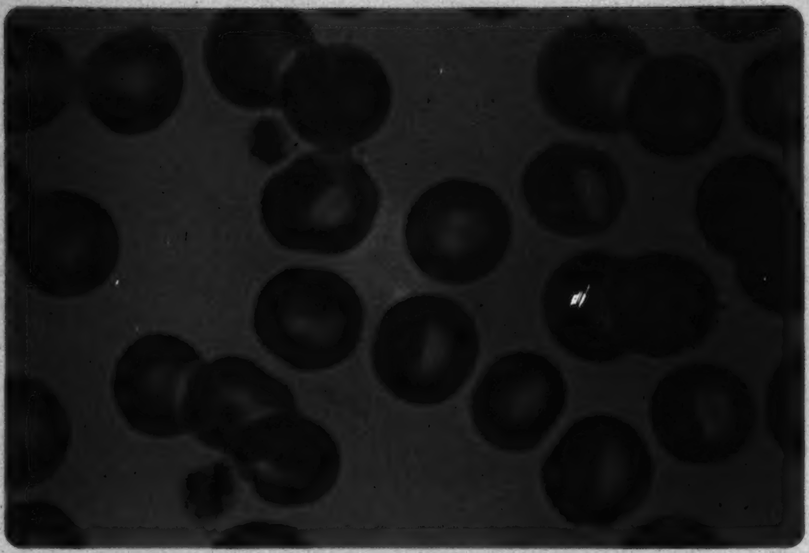


PLATE I.

PATIENT E.H. PERIPHERAL SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 100

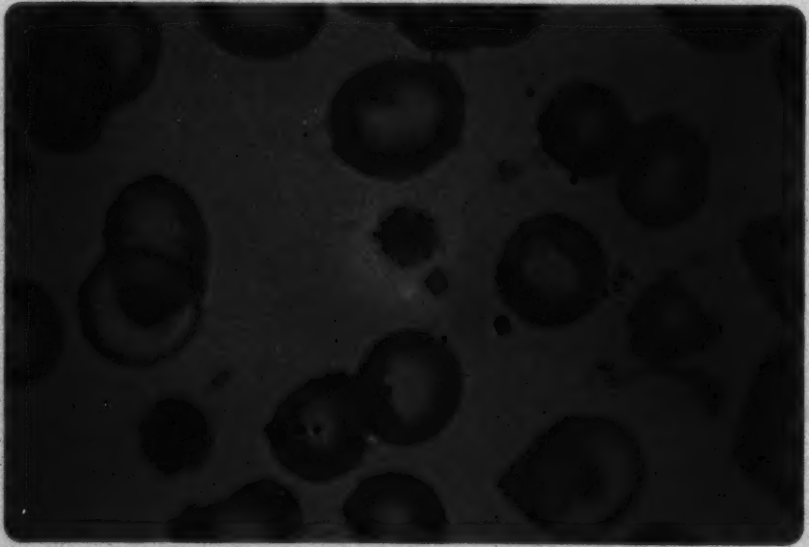


PLATE II.

PATIENT K.M. PERIPHERAL SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 1000

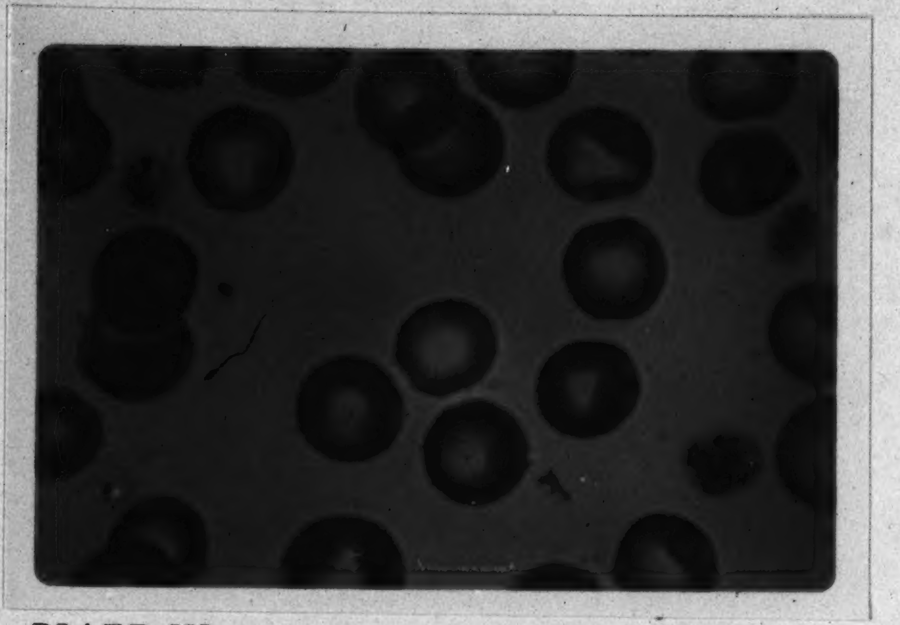


PLATE III.

PATIENT A.N. PERIPHERAL SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 1,000

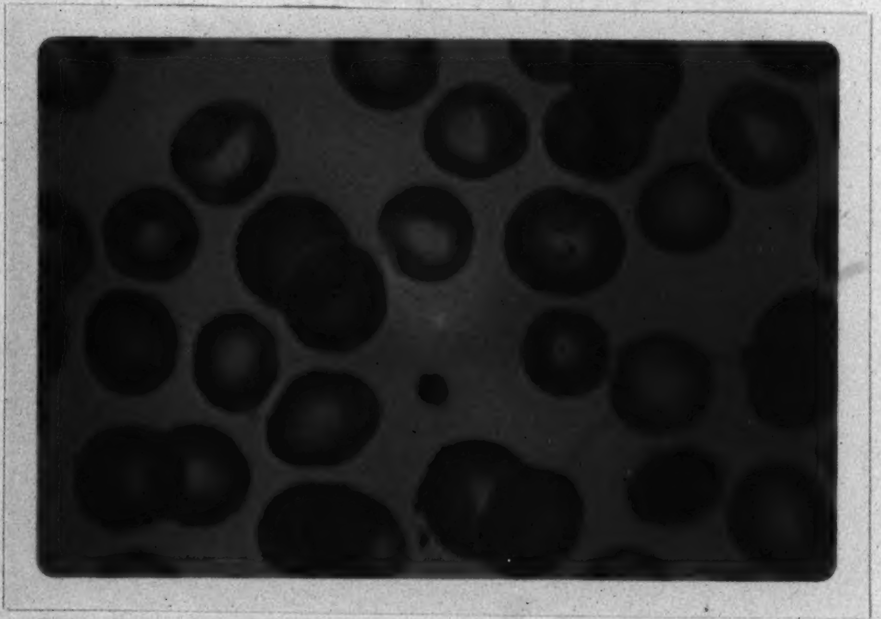


PLATE IV.

PATIENT J.R. PERIPHERAL SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 1000

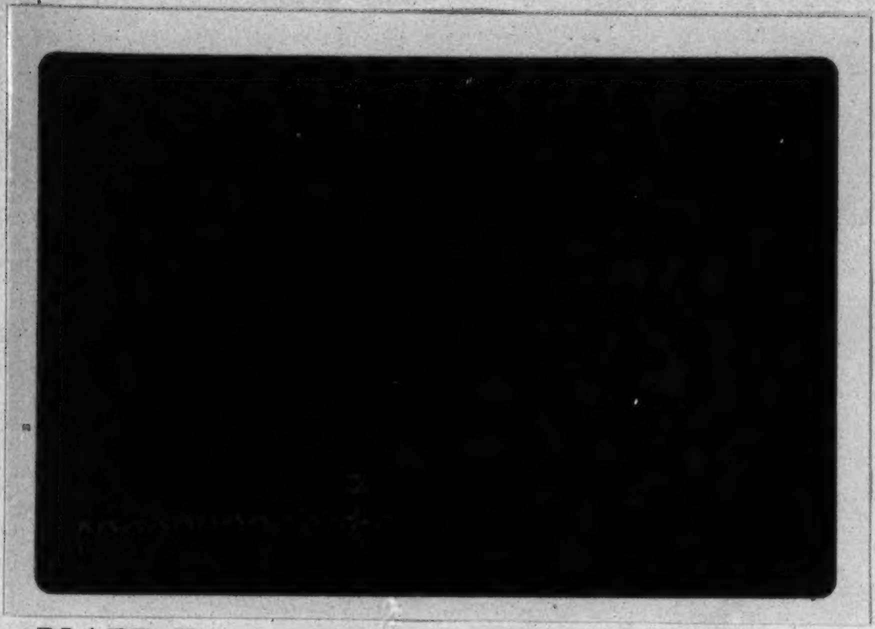


PLATE V.

PATIENT J.L. PERIPHERAL SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 200

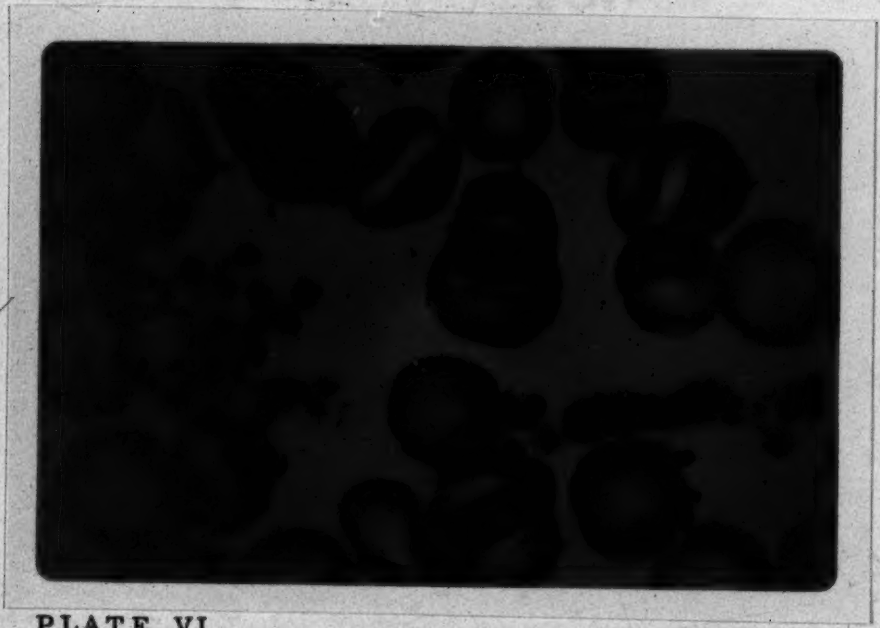


PLATE VI.

PATIENT J.L. PERIPHERAL SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 1,000

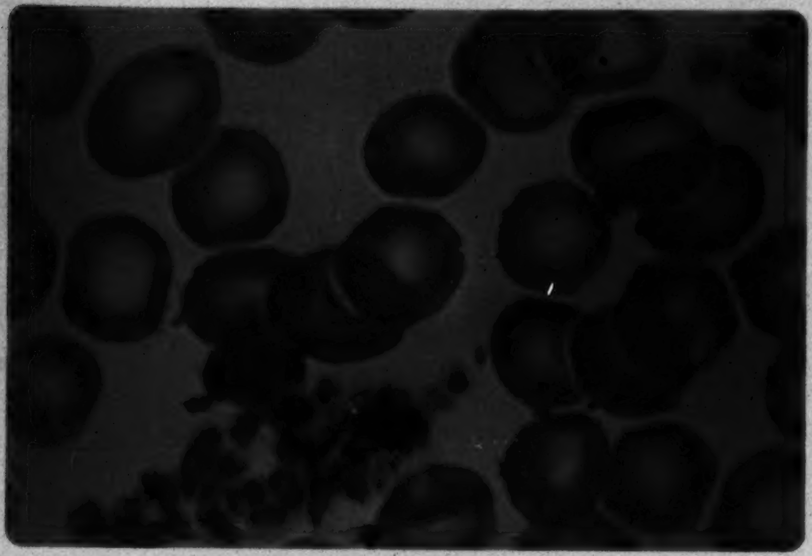


PLATE VII.

PATIENT J.L. PERIPHERAL SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 1,000

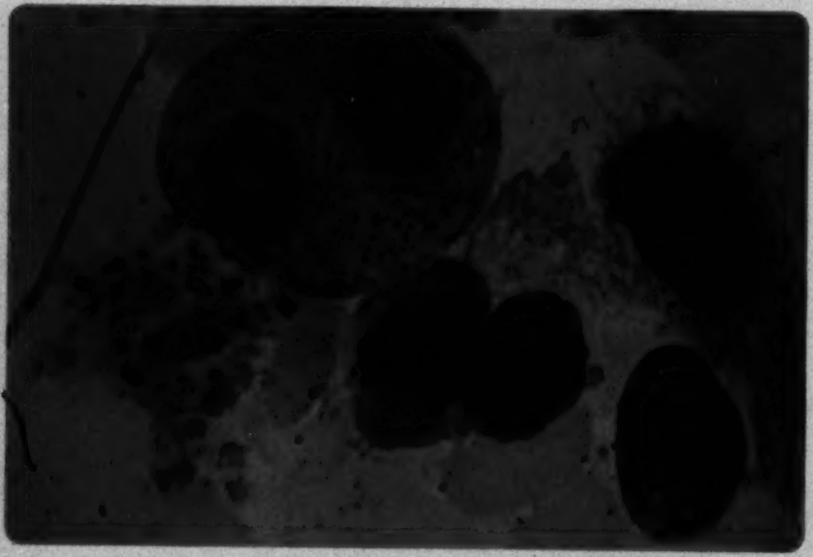


PLATE VIII.

PATIENT J.L. BONE MARROW SMEAR
MAY - GRÜNWALD - GIEMSA STAIN x 1000

BONE MARROW MORPHOLOGY:

Sullivan et al[56] showed a marked reduction in megakaryocytes in alcoholics immediately after admission. Five days later they noticed a pronounced increase in megakaryocytes with young forms predominating. They suggest that thrombocytopenia in alcoholics is a direct effect of alcohol upon megakaryocyte maturation and is not due to increased platelet destruction.

Nine of ten of our alcoholics had normal megakaryocytes on bone marrow aspirations which were performed one to six days following admission (Table II). The tenth patient (#4 G.P.) was difficult to assess because of a sparse overall cellularity. The megakaryocytes were thought to be probably reduced in number.

Also, in spite of the fact that one patient (#10 C.H.) had normal megakaryocytes, his platelet count was still low (116,000/cmm) five days after admission and stayed below normal levels for ten days.

It would seem therefore that a disturbance in thrombopoiesis is not the only mechanism involved in producing thrombocytopenia in the alcoholic patient although it cannot be ignored as having some influence. The one patient who had his bone marrow performed within 24 hours of his admission was the only one showing a possible decrease in megakaryocytes. However, even patients who had their bone marrow aspirated three days after admission had plentiful megakaryocytes, whereas Sullivan did not find an increase in megakaryocyte numbers until five days following alcohol withdrawal.

TABLE II: - ABUNDANCE OF BONE MARROW MEGAKARYOCYTES

Patient		Platelet Count on Admission /cmm	Platelet Count at time of Bone Marrow /cmm	No. of Days Following Admission	Pathologist's Comments
1.	Z.C.	67,000	143,000	6	Megakaryocytes Normal
2.	J.L.	43,000	164,000	4	Megakaryocytes Plentiful; Many Show Normal Adult Morphology with Granular Cytoplasm.
3.	A.M.	47,000	120,000	5	Megakaryocytes Normal.
4.	G.P.	100,000	72,000	1	Compatible with Starvation Marrow; Megakaryocytes Probably Reduced in Number.
5.	H.M.	119,000	143,000	5	Megakaryocytes Present in Normal Numbers.
6.	A.N.	122,000	171,000	5	Megakaryocytes Probably Normal.
7.	J.R.	98,000	79,000	4	Megakaryocytes Normal in Number.
8.	E.H.	97,000	142,000	3	Megakaryocytes Plentiful and Appear to be of Normal Morphology.
9.	K.M.	106,000	Not Done	3	Normal Mature Megakaryocytes.
10.	C.H.	90,000	116,000	5	Megakaryocytes Present in Normal Numbers.

PLATELET SURVIVALS :

Cowan demonstrated decreased survival of autologous platelets in acute alcoholics after admission to the hospital[57,58]. The half-life ($T\frac{1}{2}$) ranged from 23 to 75 (average 49) hours and correlated roughly with the platelet count. Our patients showed a similar correlation between the Cr^{51} platelet half-life and the platelet count at the time the survival was initiated (Fig. 17) ($r = 0.82$ $p<0.01$)[49]. Similarly, it was noted that there was a correlation between the platelet half life and the length of time following admission that the platelet survival was started (Fig. 18) ($r = 0.65$ $p<0.05$)[49].

Platelet survivals were therefore considered in one of three groups: (i) normals; (ii) alcoholics whose platelet survivals were started within three days of admission to hospital; and (iii) alcoholics whose platelet survivals were started five to six days following admission. Normal half-lives varied from 3 to 4.2 days, with a mean of 3.8. (Fig. 19 a & b). Mean values for the alcoholics whose platelet survivals were started five to six days following admission did not differ from those of the normals. (Wilcoxon two sample test $p > 0.2$ for each day) (Fig. 20)[42]

The seven alcoholics whose platelet survivals were initiated within three days of admission to hospital had half life values of 2.2 to 3.3 days (mean 2.6 days) (Fig. 21 a & b). Their survival curves differed significantly

PLOT TO SHOW THE RELATIONSHIP BETWEEN THE PLATELET HALF-LIFE (AS DETERMINED BY Cr^{51} AUTOLOGOUS PLATELET SURVIVALS) AND THE PLATELET COUNT ON THE DAY ON WHICH THE SURVIVAL WAS INITIATED

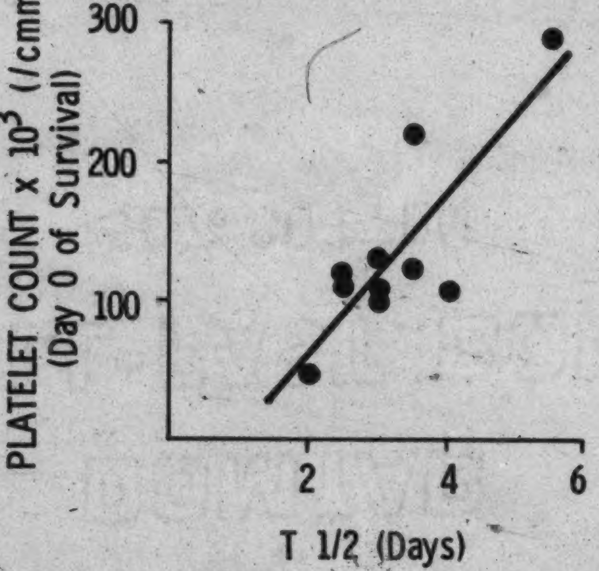


Fig. 17

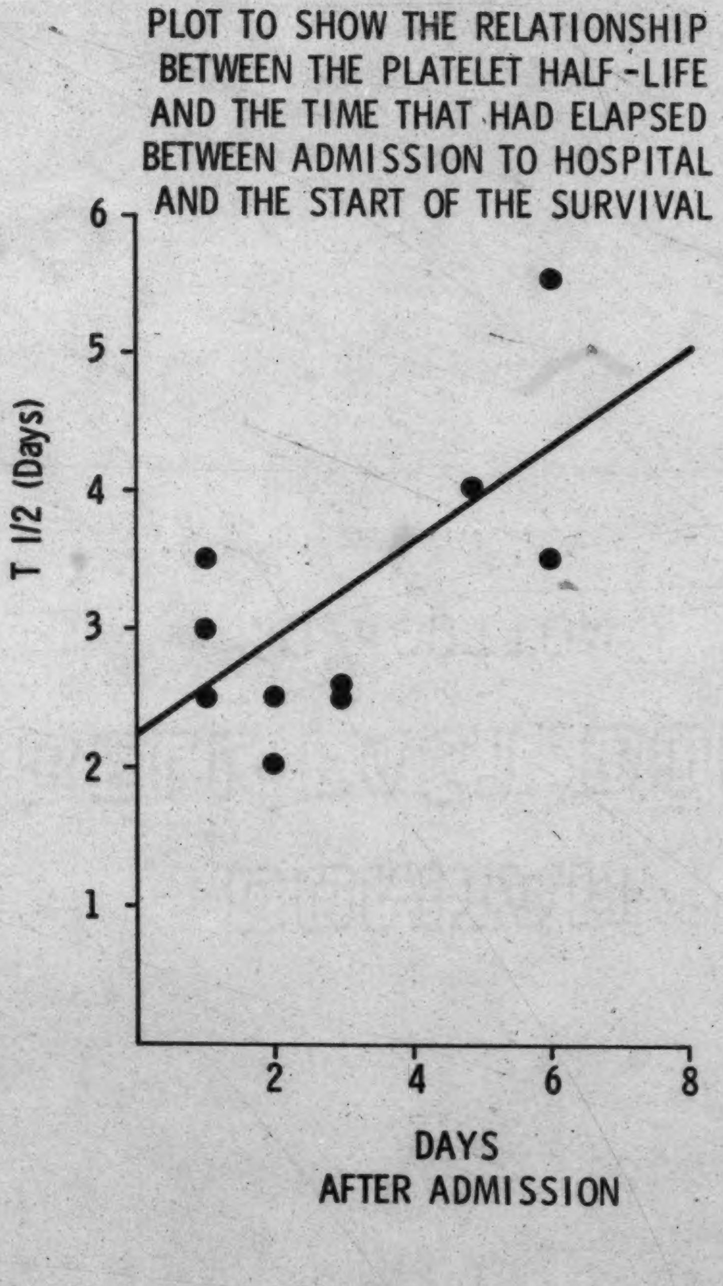
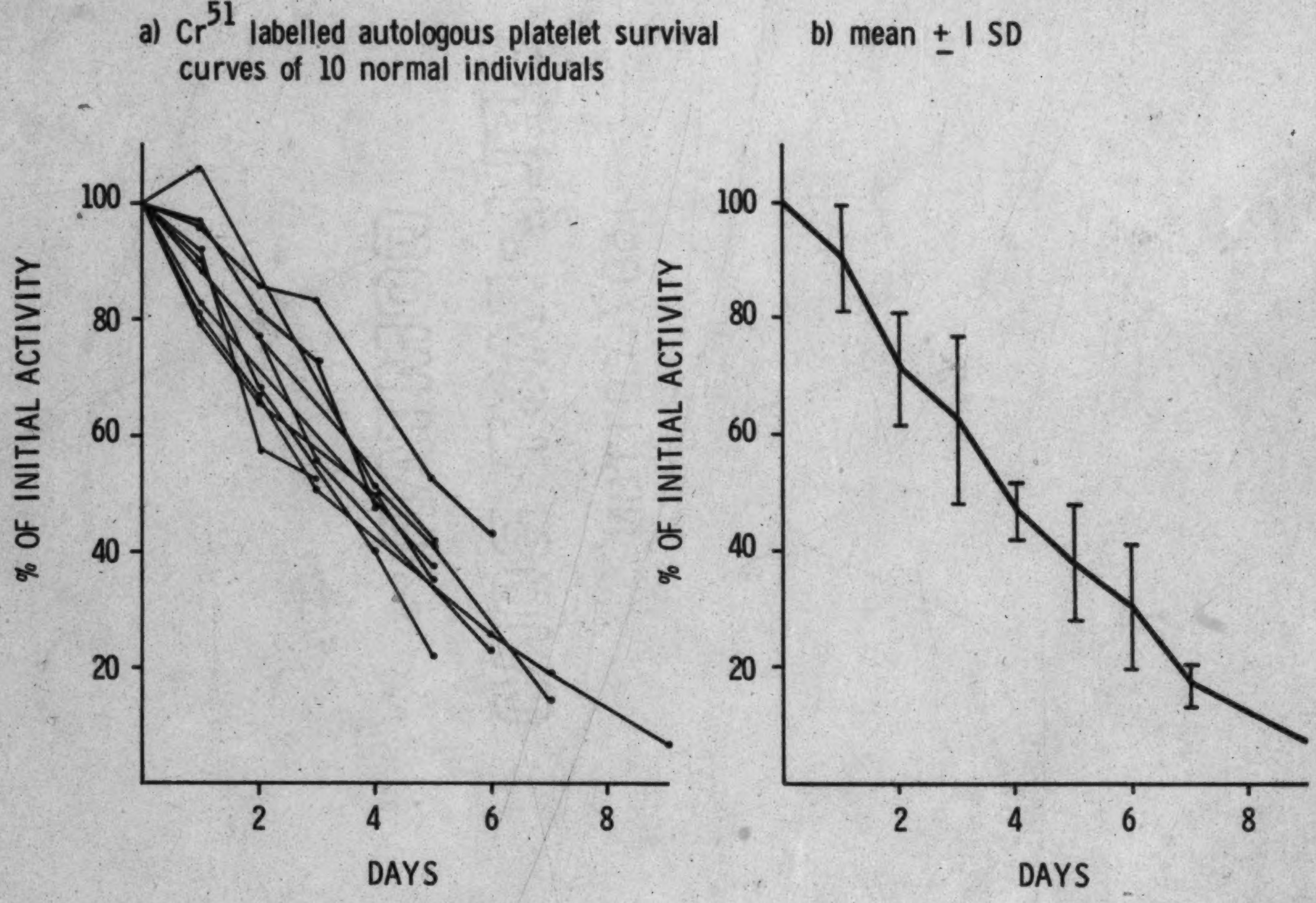

PLOT TO SHOW THE RELATIONSHIP BETWEEN THE PLATELET HALF-LIFE AND THE TIME THAT HAD ELAPSED BETWEEN ADMISSION TO HOSPITAL AND THE START OF THE SURVIVAL
T 1/2 (Days)
6
5
4
3
2
1
2
4
6
8
DAYS
AFTER ADMISSION

Fig. 18



a) Cr^{51} labelled autologous platelet survival curves of 10 normal individuals

b) mean ± 1 SD

Fig. 19

SURVIVAL OF Cr^{51} LABELLED AUTOLOGOUS PLATELETS IN NORMAL INDIVIDUALS (•——•) AND IN ALCOHOLICS WHOSE PLATELET SURVIVALS WERE STARTED 5-6 DAYS FOLLOWING ADMISSION (•—·—•)

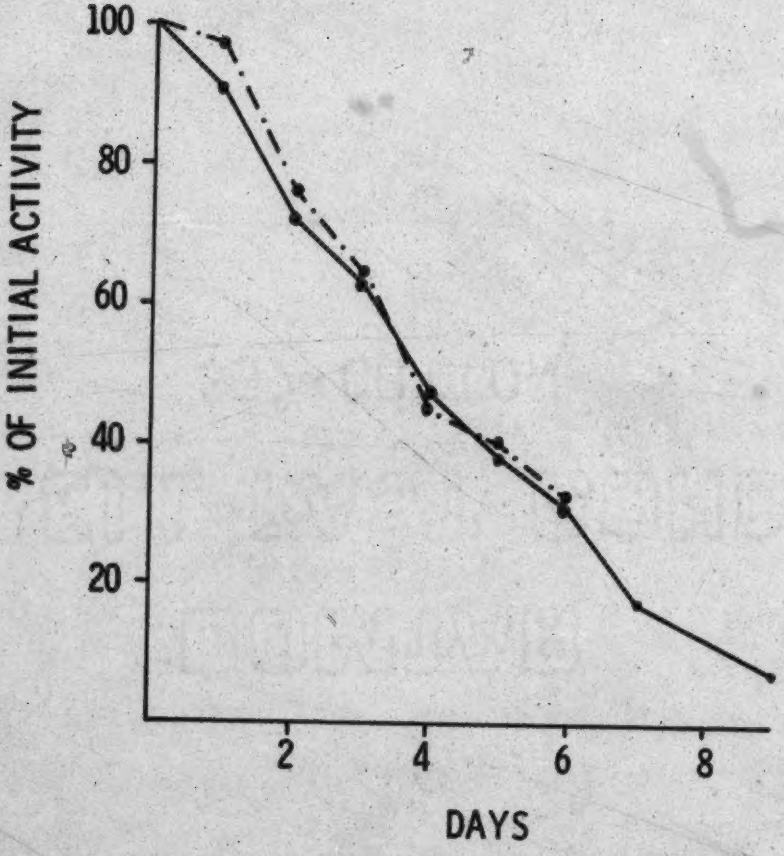
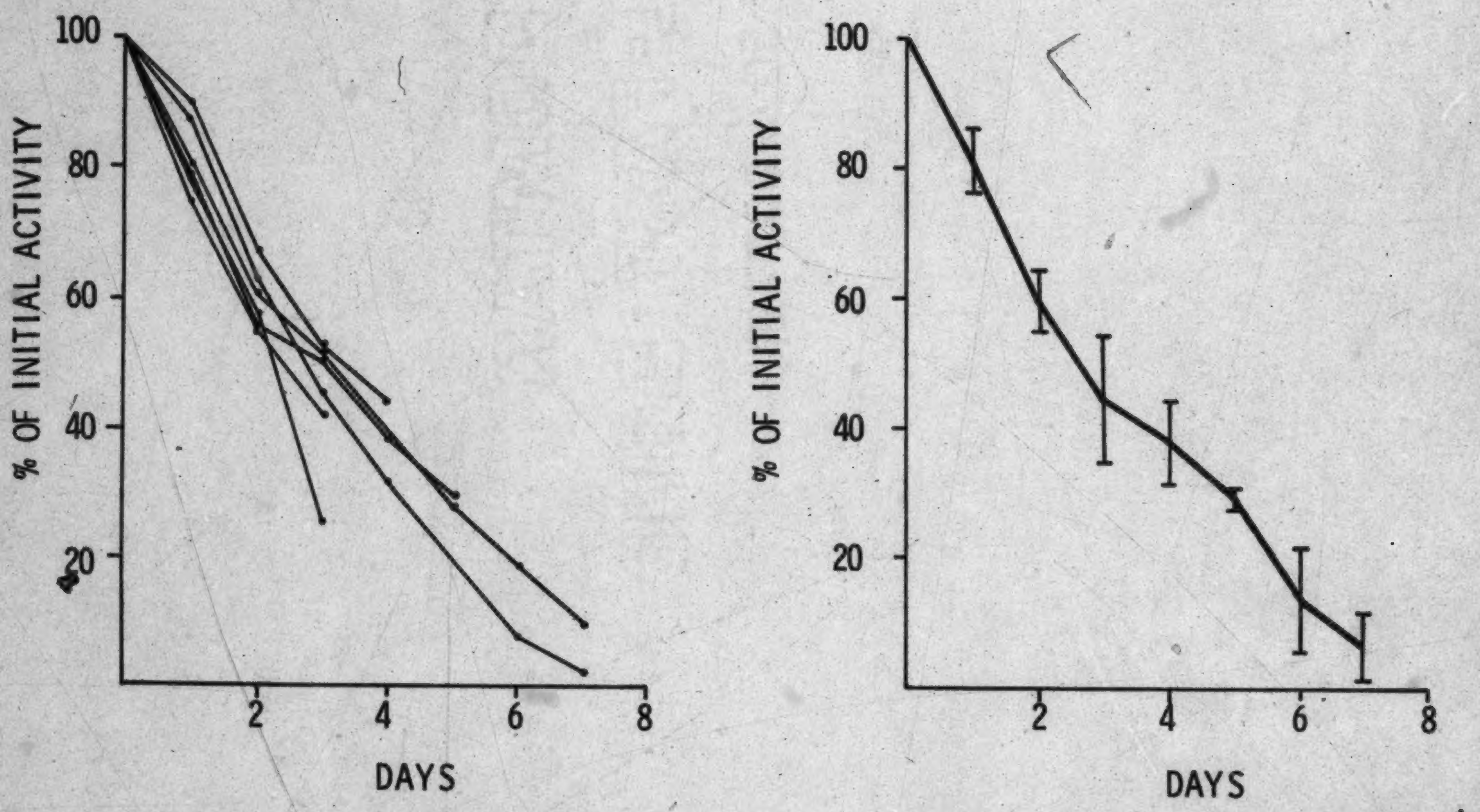


Fig. 20



a) Cr^{51} labelled autologous platelet survival. Survival curves of 7 alcoholic patients whose platelet survivals were started within 3 days of their admission to hospital

b) Mean ± 1 SD

Fig. 21

from that of the normals at Days 1 ($p = 0.014$), 2 ($p = 0.017$), and 3 ($p = 0.015$) only. (Wilcoxon two sample test) (Fig. 22).

SURFACE COUNTING :

There was no evidence of increased splenic sequestration. Spleen: heart ratios in the alcoholic patients ranged from 0.51 to 1.95 (mean 0.94) as compared with ratios of 0.99 to 1.54 (mean 1.19) in the normal individuals. Similarly, liver:heart ratios ranged from 0.52 to 0.82 (mean 0.67) in the alcoholics as compared with 0.60 to 1.36 (mean 0.89) in the normals.

Cr^{51} labelled autologous platelet survival curves of 10 normal individuals (⊙—⊙) and 7 alcoholic patients (•—•) whose platelet survivals were started within 3 days of their admission to hospital. There is a statistical difference between the values at Days 1,2,3 ($p<0.02$) but none between those of Days 4 to 7 ($p>0.05$)

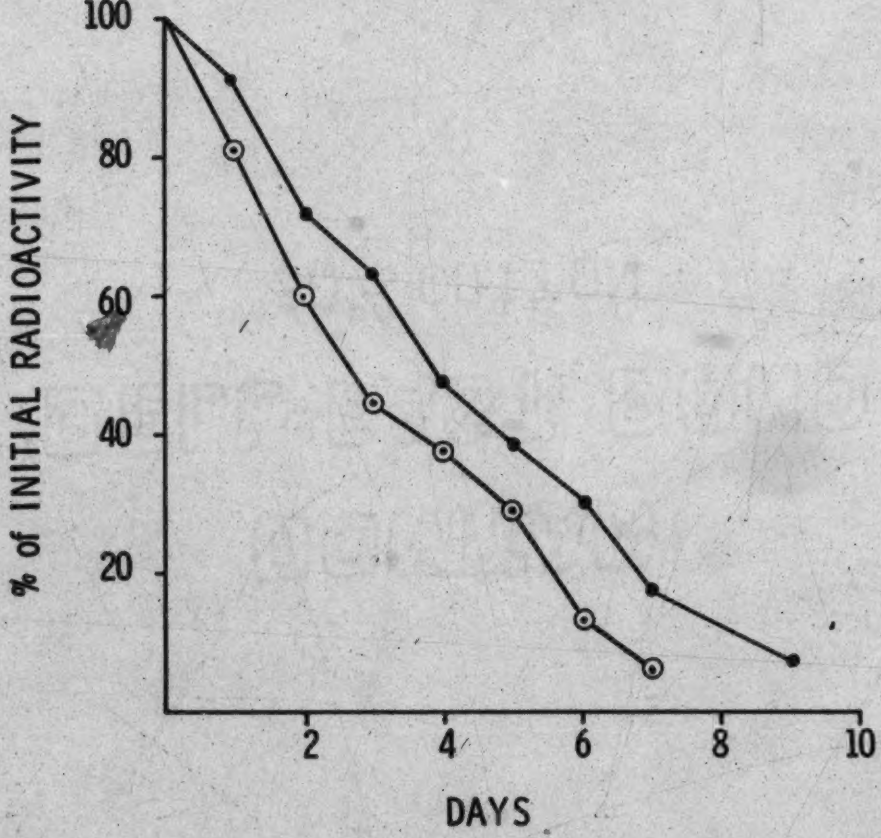


Fig. 22

CHAPTER IV

CONCLUSIONS

No correlation could be found between the patient's platelet count on admission and his dietary history or type of alcoholic intake prior to admission. However, only those patients who had been drinking steadily for five or more days were thrombocytopenic which would seem to indicate that a certain period is required before a sustained fall in the platelet count occurs.

There was no correlation between the platelet count and other hematologic parameters or liver function tests.

All patients had increases in their platelet counts, starting one to three days following admission. At the same time increased numbers of large platelets were evident on their peripheral smears.

Survival of Cr^{51} labelled autologous platelets in alcoholic patients immediately following withdrawal from alcohol was significantly different from that of normal individuals at Days 1, 2 and 3 following the start of the survival.

Cowan demonstrated that the survival of normal homologous platelets in a thrombocytopenic alcoholic recipient was intermediate between his control survival (during abstinence) and that of the autologous platelets during ingestion of alcohol. He suggested, therefore, that both intracorpuscular and extracorpuscular factors are responsible for the decreased lifespan of circulating platelets in alcoholic patients. The increased disappearance of platelets seen in the first three days in alcoholic patients as compared with normals in our study could represent one population of platelets still affected by alcohol while those living a normal lifespan after three days represent a second population of normal platelets produced after alcohol was withdrawn and no longer affected by its toxic influence in the circulation. This would be compatible with the appearance of young "large" platelets in the circulation following the withdrawal of alcohol.

A succession of events in the establishment of thrombocytopenia in the alcoholic patient can be seen. First, there is a brief, transient fall in the platelet count occurring within six hours of the onset of drinking alcohol and returning to normal levels within 24 hours. There is no concurrent rise in splenic radioactivity when Cr^{51} - labelled autologous platelets are studied. A second, sustained fall in the platelet count occurs approximately five to seven days later if drinking is continued. This fall is unrelated to the patient's liver function and may occur without concomitant folate deficiency or other hematologic abnormalities. The platelet count remains depressed as long as the individual is drinking alcohol. With the cessation of alcohol a rise in the platelet count occurs, returning to normal values within two to seven days. This rise is associated with the appearance of increased proportions of large "young" platelets in the peripheral circulation. A rebound thrombocytosis often occurs, reaching peak levels at approximately 10 days after the cessation of alcohol and then returning to normal values.

The period required both for a fall in the platelet count and its recovery to occur is compatible with an effect of alcohol on the maturation of the megakaryocytic line. Similarly the appearance of young platelets as the platelet count increases would suggest that alcohol-related thrombocytopenia is due in part at least to a suppression of thrombopoiesis.

At the same time Cr^{51} autologous platelet-survival studies indicate a decreased lifespan of circulating platelets in the presence of alcohol. The importance of both intracorpuscular and extracorpuscular factors is demonstrated by an only partially decreased lifespan when labelled homologous platelets are infused into an alcoholic recipient. There is no evidence of temporary sequestration in the spleen or liver and it would seem that the platelets are removed from circulation at some unknown site.

The extracorpuscular factor involved in the shortening of the lifespan seems most likely to be alcohol or a by-product of alcohol metabolism. The nature of the intracorpuscular factor is open to speculation and it is perhaps this factor which is involved in the early transient fall in the platelet count when drinking is initiated.

CHAPTER V

THE EFFECT OF ALCOHOL ON PLATELET AGGREGATION

INTRODUCTION :

Ryback and Desforges indicated the possibility of two separate effects when alcohol was administered to hospitalized volunteers[5]. A sustained thrombocytopenia occurred four to seven days following the onset of drinking and it is probable that this is the thrombocytopenia encountered in alcoholic patients admitted to the hospital. However, an earlier transient drop in the platelet count was also documented. This occurred at four to six hours after intravenous infusion of ethanol was started and returned to pre-infusion levels within twenty-four hours[1,3]. When Cr^{51}-labelled platelets were present, platelet radioactivity paralleled the fall in the platelet count but there was no concurrent increase in hepatic or splenic radioactivity.

It would appear that a mechanism different from that causing a sustained thrombocytopenia is involved in this early transient fall. First, because of the short time interval in which it takes place (as opposed to the period of at least a week required to cause a sustained thrombocytopenia) and secondly, because of the rapidity with which the platelet count returns to normal (24 hours as opposed to an average of five to eighteen days[4] following a long-term binge).

Ryback and Desforges suggest that this transient fall may be related to platelet aggregation possibly causing sequestration of the circulating platelets at some unknown site.

Ethanol is known to cause changes in both epinephrine[7] and serotonin[6] metabolism in man, probably related to the increased NADH:NAD ratio effected by ethanol metabolism[59,60]. Although these metabolites are both involved in platelet aggregation the effect of alcohol on them is not transient and it therefore seems unlikely that this is the mechanism involved.

Studies were therefore undertaken to examine the in vitro effect of alcohol on platelet aggregation.

MATERIALS :

Platelet aggregation was studied in the Payton Single-Channel Aggregation Module coupled with a Bausch and Lomb VOM 5 Recorder*[, 61]. Platelet aggregation is assessed photometrically in a temperature controlled, constantly stirred, siliconized system and variations in light transmittance are displayed graphically on the recording system. As the platelets aggregate, more and more light is allowed to contact the photo-conductive cell producing in turn a positive deflection of the pen on the recorder. The upward sweep of the pen is not smooth but is interrupted by vertical oscillations as the aggregates being stirred about modulate the light beam. These oscillations are roughly proportional to the size of the aggregates.

---

* Payton Associates Ltd., Scarborough, Ont.

Siliconized glassware or plastic equipment was used throughout the aggregation studies. Samples were stirred in a 0.312 inch diameter siliconized glass curette with a horizontally placed siliconized stir bar. Samples were maintained at a temperature of 37° C (± 0.1° C) and magnetically stirred at a speed of 1000 rpm (± 20 rpm). Normally 0.05 ml of test solution was added to 0.5 ml PRP.

4.5 ml whole blood was withdrawn into siliconized vacutainer tubes containing 0.05 ml 3.8% Sodium Citrate* and centrifuged at 800 rpm (170G) for 10 mins. at room temperature[62]. The supernatant platelet rich plasma was removed to a small plastic tube** and kept at room temperature.

The Module was calibrated daily to a standard deflection of 9 mV between platelet rich plasma and distilled water. The chart speed was set at 1" per minute.

* Becton, Dickinson & Co. Rutherford, N.J., No. 3206. Each tube also contains 0.2 mg/ml Potassium Sorbate as an Antimycotic Agent.

** Falcon Plastics, Los Angeles, Calif.

Reagents were made up and diluted in Tyrode's buffer which was freshly prepared before each test. A stock solution of 16 gms% NaCl, 0.4 gm% KCl, 2 gms% $NaHCO_3$ and 0.1 gm% NaH_2PO_4 was kept in the refrigerator. This was stable for 2 - 3 months. Tyrode's buffer was prepared from this by adding 1 gm of dextrose to 50 ml of stock solution and adjusting the volume to 100 ml with distilled water. The pH was then adjusted to 7.35 with HCL or NaOH.

Adenosine-5-diphosphate (Disodium salt)* was stored at -4° C for up to two to three months, as a 10^{-2} Molar solution and diluted before use to 0.5×10^{-4}M to achieve a final concentration of 4.5 μM.

Adrenalin was obtained as a 100 mgs% solution of Adrenalin Chloride** and diluted before use to 5 mg% solution to achieve a final concentration of 2.48×10^{-5}M.[63]

EFFECT PRODUCED BY ADP AND ADRENALIN IN HUMAN CITRATED PLASMA :

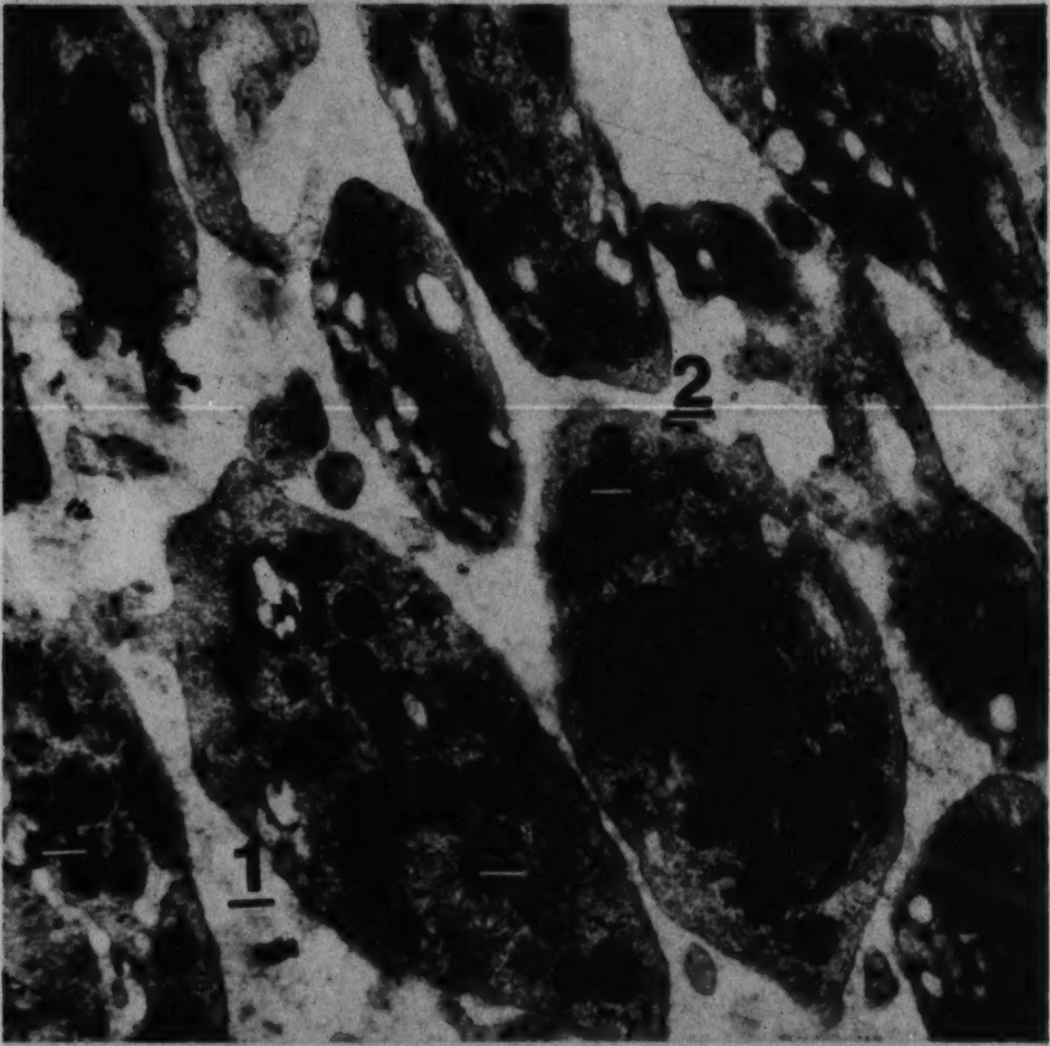
Following the addition of either ADP or adrenalin to citrated PRP in the aggregation module there is a rapid conversion of disc-shaped platelets (Plates IX and X) to irregular ovals and spheres (Plates XI and XII).

---

* Sigma Chemical Co., St. Louis, Mo.

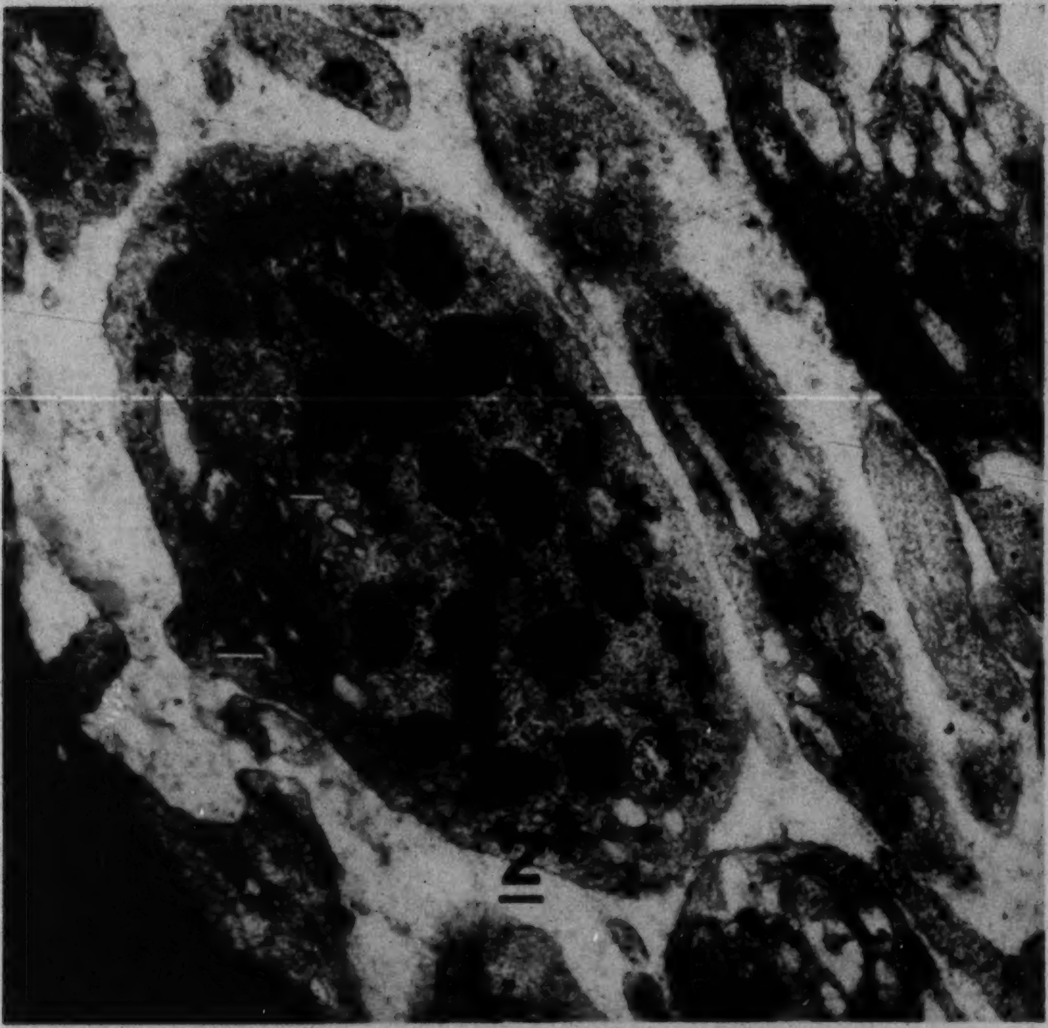
** Parke, Davis and Company, Ltd., Brockville, Ont. Epinephrine Hydrochloride dissolved in isotonic sodium chloride solution with not more than 0.1% sodium disulphite as a preservative.

PLATE IX



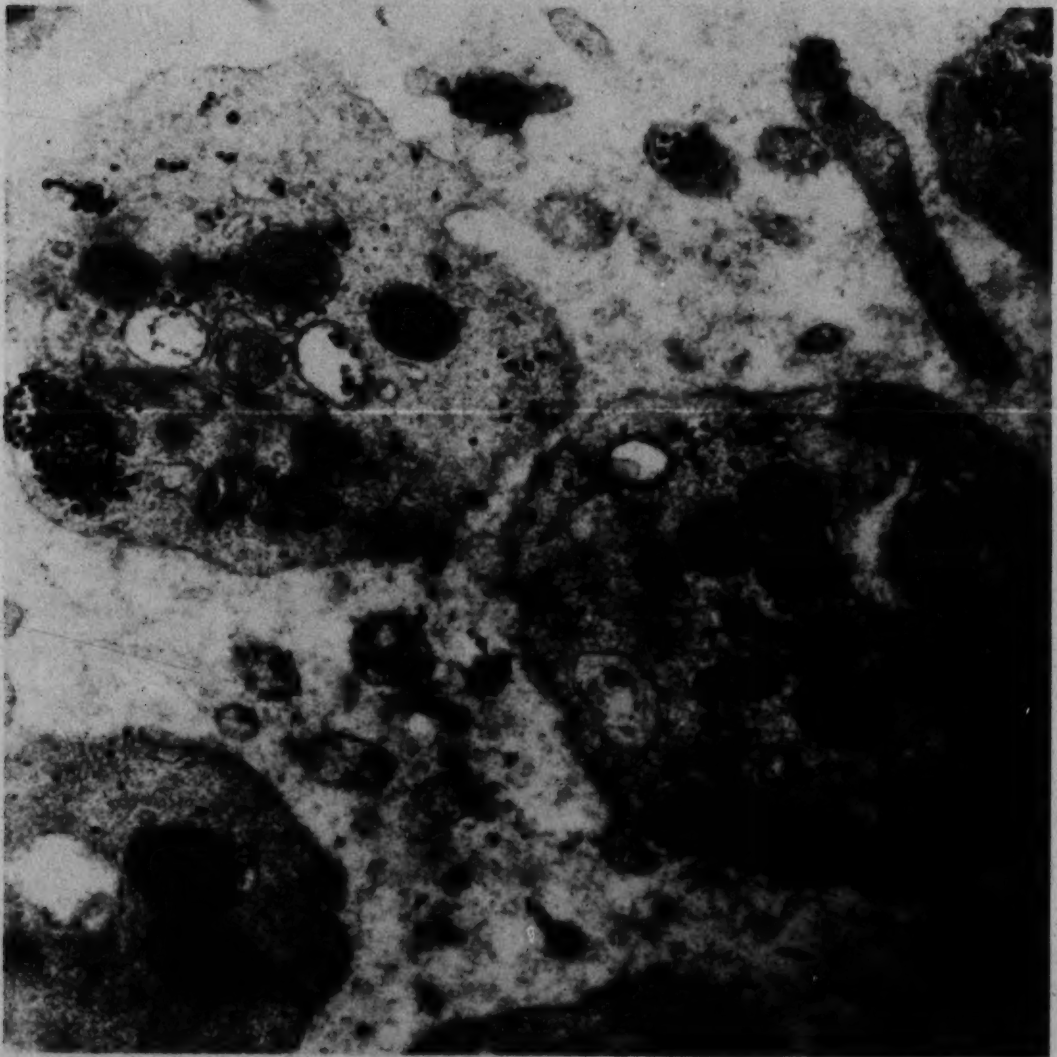
Platelets prior to aggregation (x 42,000). Surface connecting system (1), cross-sectioned microtubles (2), mitochondrion (3), very dense body (4), granule (5). Glutaraldehyde-osmium fixation.

PLATE X



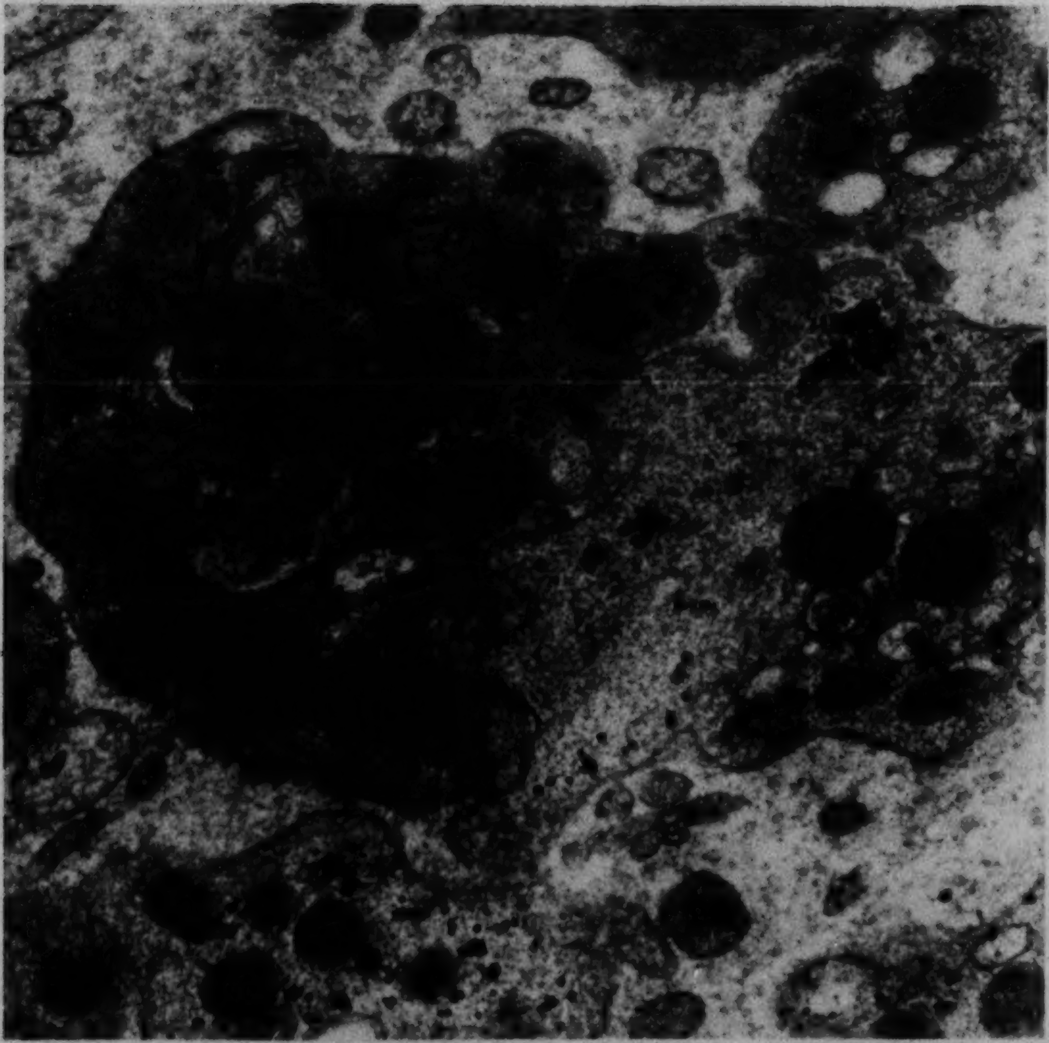
Platelets prior to aggregation (x 55,000). Golgi apparatus (1), glycogen granules (2). Glutaraldehyde-osmium fixation.

PLATE XI



Platelets exhibiting shape change prior to aggregation (x 54,000). Glutaraldehyde-osmium fixation.

PLATE XII



Platelets exhibiting shape change prior to aggregation (x 96,000).
Glutaraldehyde-osmium fixation.

This results in an initial decrease in light transmission on the recording apparatus[64] (Section a. Fig. 23). Clumping of these changed platelets results in a subsequent increase in light transmission. This sequence of changes produces a "hump" tracing, (Section b. Fig 23) which is the primary phase of aggregation and occurs as a direct result of the addition of the aggregating agent. All of these phenomena (i.e. early shape change, swelling and the initial wave of platelet clumping) are completely reversible[65].

The second wave of aggregation is associated with the secretion (or release) by the platelets of substances known to be confined to specific organelles. These substances include serotonin, ADP, ATP, hydrolytic enzymes, cationic proteins and mucopolysaccaride. The products of secretion must reach a sufficient concentration late in the primary phase of aggregation or in the trough between the 1st and 2nd wave (Section c Fig 23) to drive the process into the irreversible stage. Hardisty, et al found that a minimum concentration of 1.2 - 1.4 μM ADP or 0.1 - 10 μM adrenalin was required in order for an irreversible secondary wave of aggregation to be initiated[66].

The mechanism involved in the extrusion of platelet organelles is not clearly understood. During early platelet response most of the granules and some dense bodies are shifted into close association in the centre of the platelet (Plate XIII). Early in the second phase of aggregation most of the dense bodies disappear from the platelets. Later, in the process of

PRIMARY AND SECONDARY AGGREGATION IN HUMAN CITRATED PLATELET RICH PLASMA PRODUCED BY ADRENALIN
(Final Concentration 2.5 x 10^{-5} M)

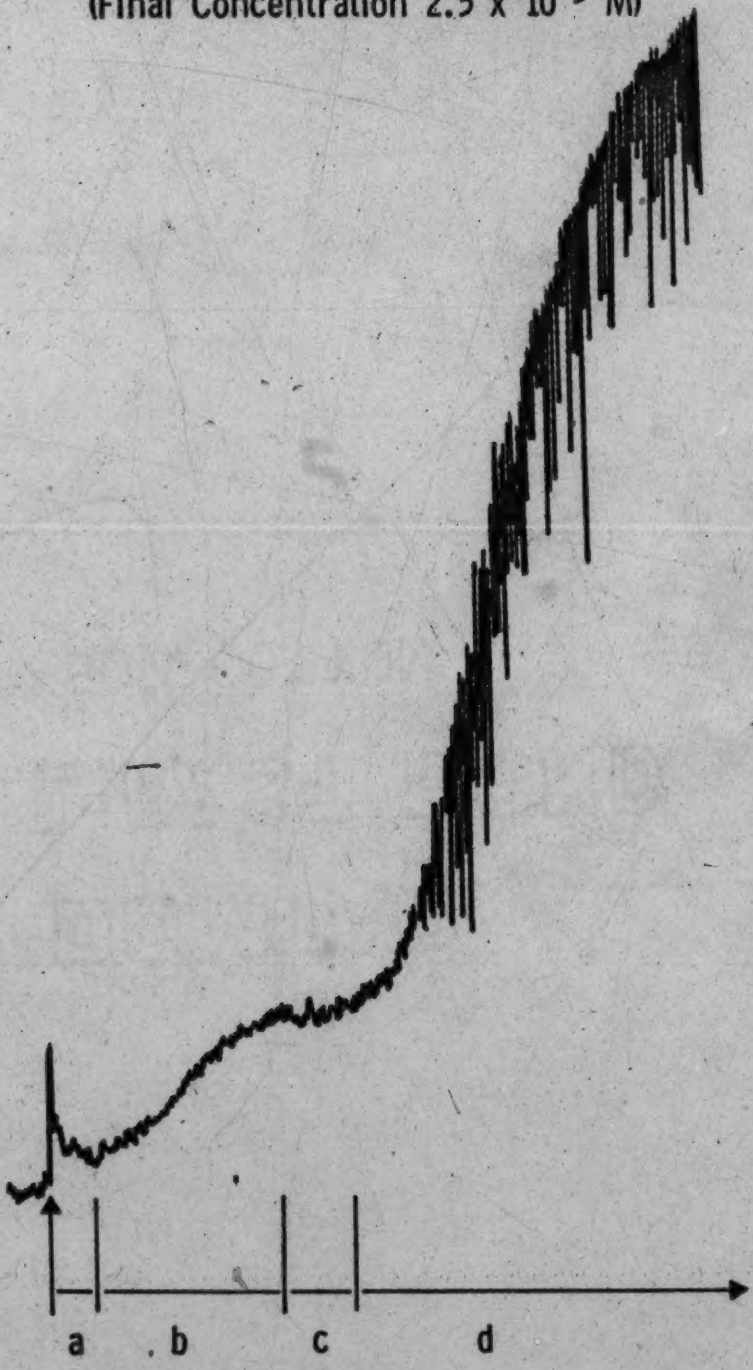
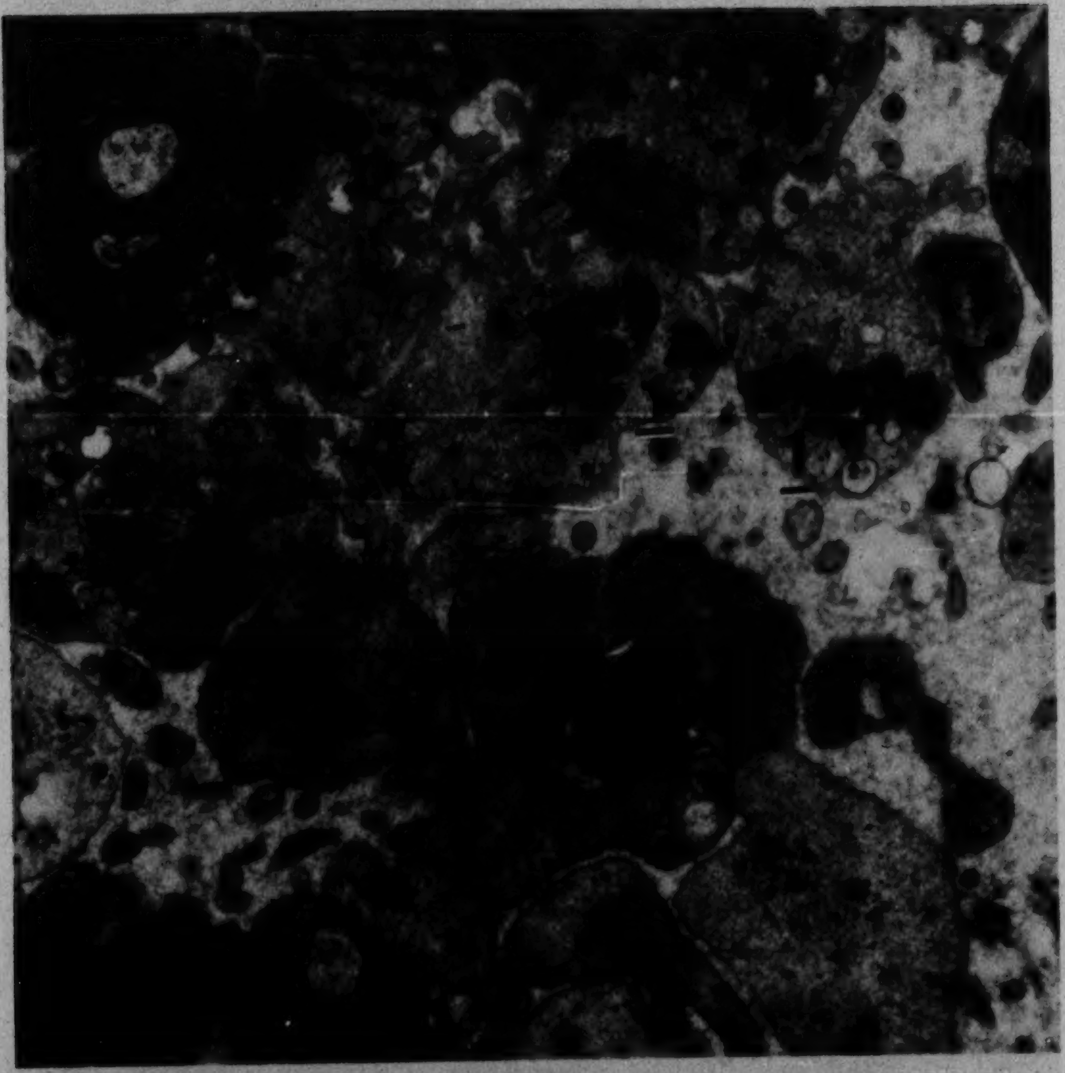


Fig. 23

PLATE XIII



Centralization of platelet granules (1) prior to aggregation (x 35,000).
Glutaraldehyde-osmium fixation.

irreversible aggregation (Section d, Fig. 23) the centrally compacted granules fuse and gradually disappear[65, 67]. Their extrusion is thought to occur through the open channel or surface connecting system and to be associated with the contraction of large masses of aggregated platelets.

QUANTITATION OF PLATELET AGGREGATION :

Platelet aggregation curves were measured according to the method of O'Brien et al [68] (Fig. 24).

A. - AMPLITUDE

The depth of the oscillations is roughly proportional to the size of the aggregates. This was measured at the peak of the primary wave of aggregation and expressed as the number of chart units.

B. - CHANGE IN LIGHT TRANSMISSION

The change in the mean level of the tracing after two minutes was measured and expressed in chart units.

C. - THE SLOPE

The steepest part of the tracing occurred when the rate of aggregation (either primary or secondary) was maximal. A line drawn through this part of the curve was extended and the vertical distance (C) that the line covered in 1 horizontal inch was measured

QUANTITATION OF PLATELET AGGREGATION

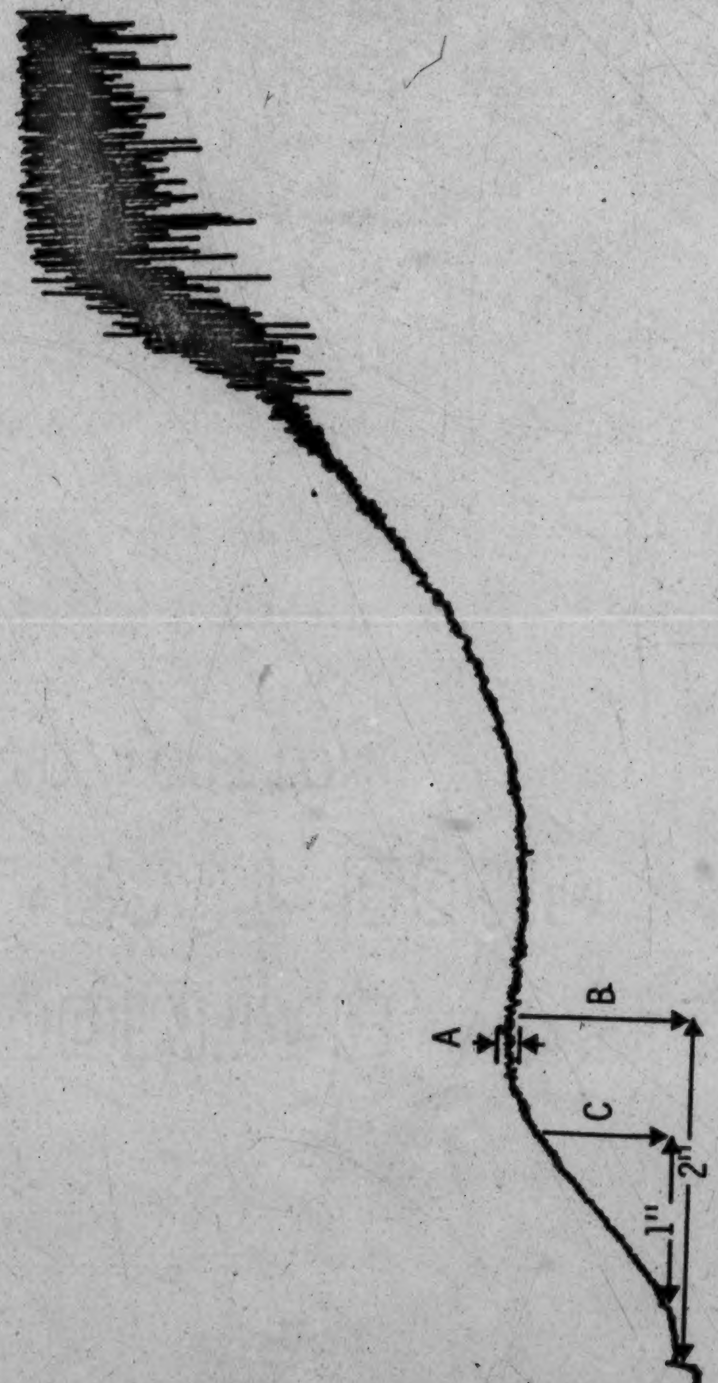


Fig. 24

in chart units. A high value indicates rapid aggregation. (Considerable variation between individuals was encountered when measuring the slopes of the secondary wave of aggregation produced by adrenalin. In order to standardize these numbers so that mean values could be calculated they have been expressed as percentage of the control value).

METHODS :

Platelet aggregation studies were performed on PRP obtained from five females and one male aged 22 to 35 years.

0.45 ml. of PRP was mixed with 0.05 ml of either: (a) 0.9% saline; or (b) 10%, 8%, 6%, 5%, 3% or 1% ethanol. (All alcohols were made up by volume in a normal saline base.) The mixtures stood at room temperature for one hour prior to aggregation with 0.05 ml of either adrenalin or APD. Any effects of aging of the PRP and aggregating agents were assessed by testing saline controls before and after each group of three ethanol concentrations. Aggregation tracings were measured as described above.

RESULTS :

Zweifler and Sanbar were unable to demonstrate any effect of 0.05% ethanol on ADP induced aggregation[69]. They measured the intensity of the aggregation as the maximum increase in light transmission and the duration of aggregation as the number of seconds required from initiation of aggregation to 50% recovery from the point of maximum rise.

Davis and Phillips, similarly measuring maximum decrease in optical density, found that the addition of ethanol to human PRP to achieve concentrations of 0.1 or 0.3% did not inhibit adenosine diphosphate-induced platelet aggregation. Nonepinephrine-induced platelet aggregation, however, was inhibited by 0.3% ethanol. [70]

In our studies a direct linear correlation was found between the concentration of alcohol in the PRP and each of the aggregation parameters that was measured. With increasing concentration the alcohol seemed to effect an overall decrease in both the rate or the duration and the intensity of platelet aggregation. This was reflected both in the individual aggregation curves and in the mean values for the six individuals as a decrease in : (i) the primary slope of aggregation (Fig. 25 and 26); (ii) the change in light transmission (Fig. 27 and 28); (iii) the amplitude of the oscillations (Fig. 29 and 30); and (iv) the slope of the secondary aggregation curve (Fig. 31 and 32).

DISCUSSION :

There has been a great deal written about defective platelet aggregation in response to stimulation by epinephrine or collagen [71-81]. This defect, however, is associated with an impairment or inhibition of release of

ADP-INDUCED AGGREGATION: SLOPE OF THE PRIMARY WAVE OF AGGREGATION vs. ETHANOL CONCENTRATION

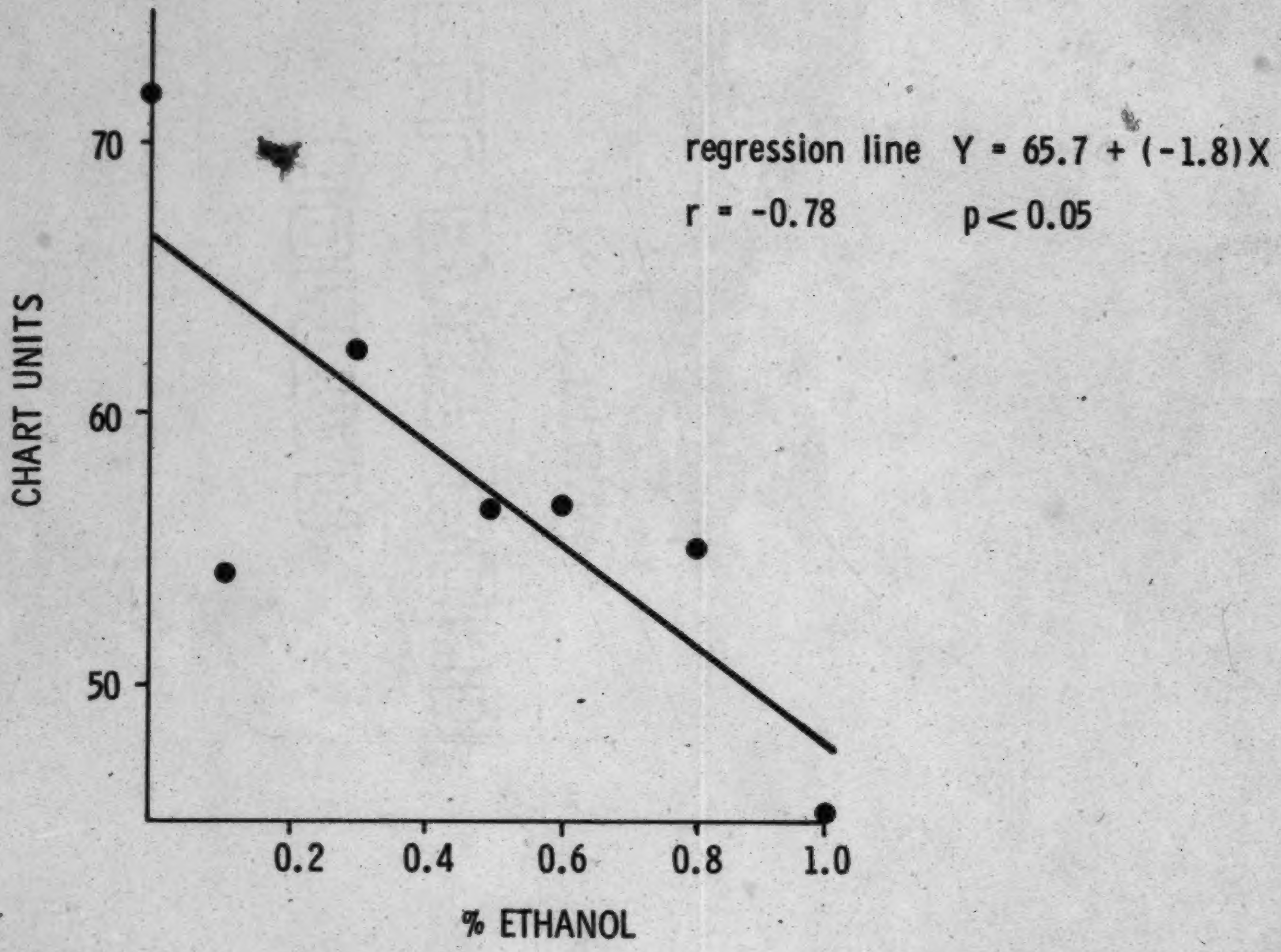
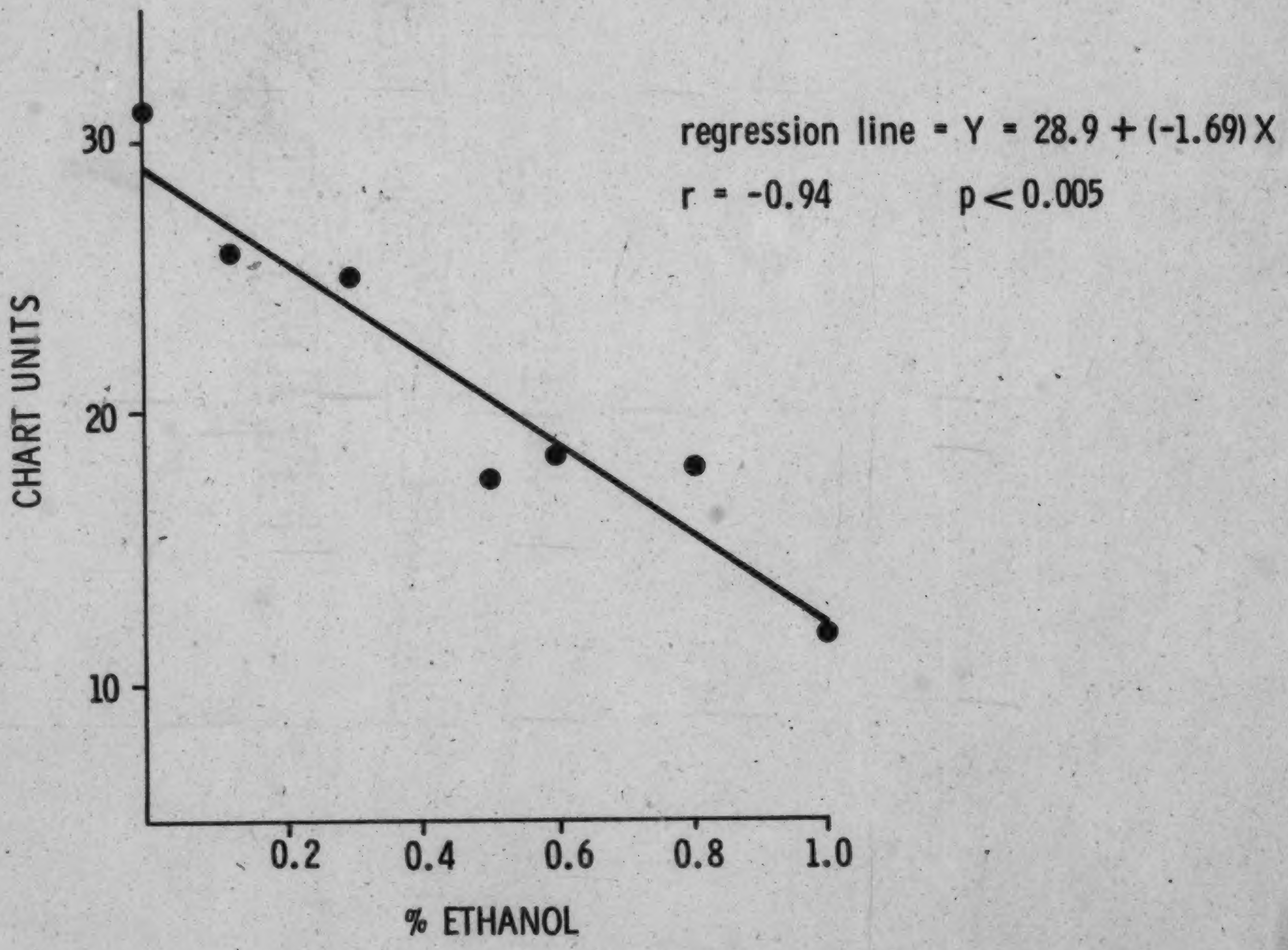


Fig. 25



ADRENALIN-INDUCED AGGREGATION:
SLOPE OF THE PRIMARY WAVE OF
AGGREGATION vs. ETHANOL CONCENTRATION

regression line = Y = 28.9 + (-1.69) X

r = -0.94 $p < 0.005$

Fig. 26

ADP-INDUCED AGGREGATION: CHANGE IN LIGHT TRANSMISSION AT 2 MINUTES vs. ETHANOL CONCENTRATION

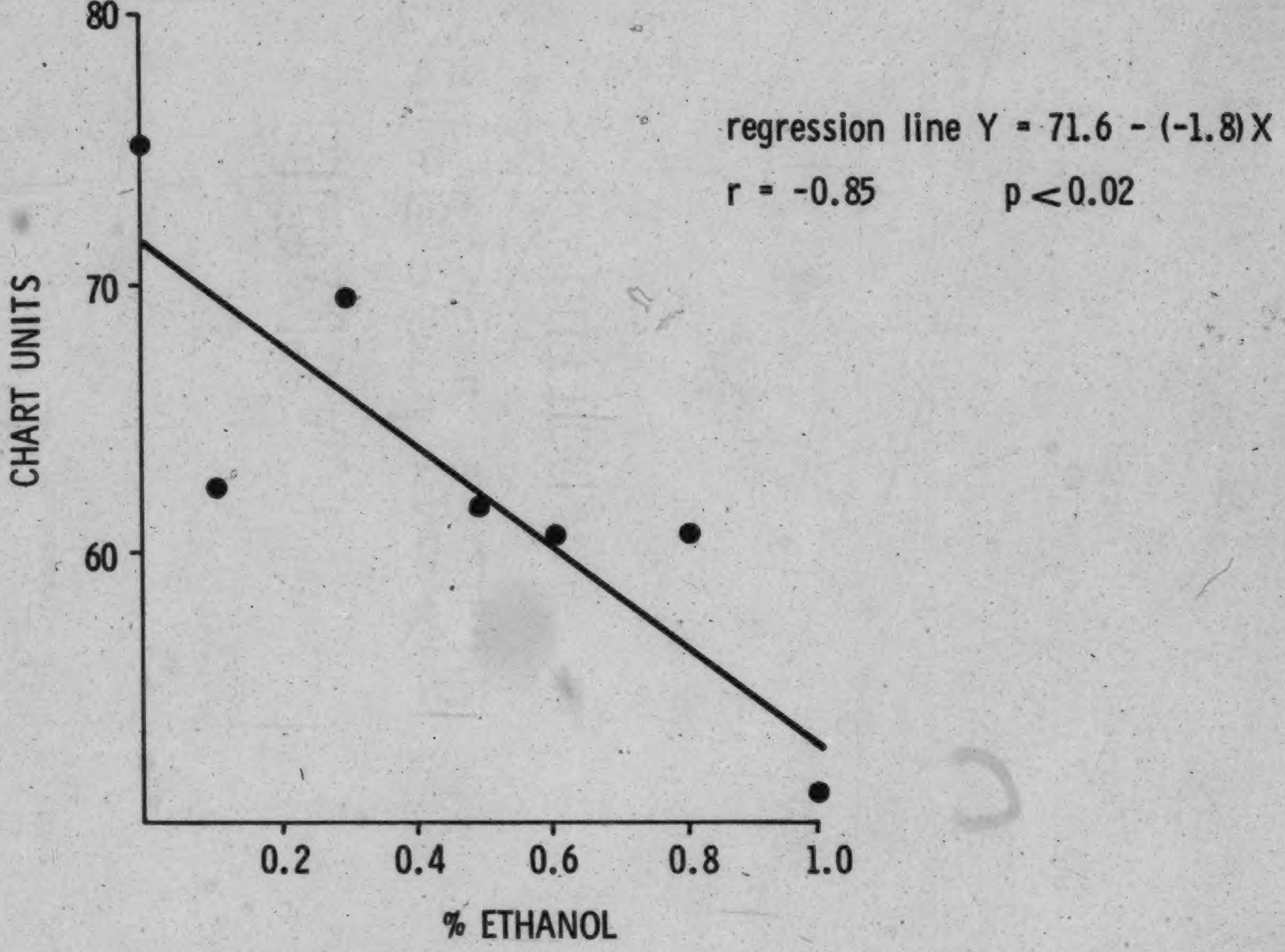


Fig. 27

ADRENALIN-INDUCED AGGREGATION:
CHANGE IN LIGHT TRANSMISSION AT
2 MINUTES vs. ETHANOL CONCENTRATION

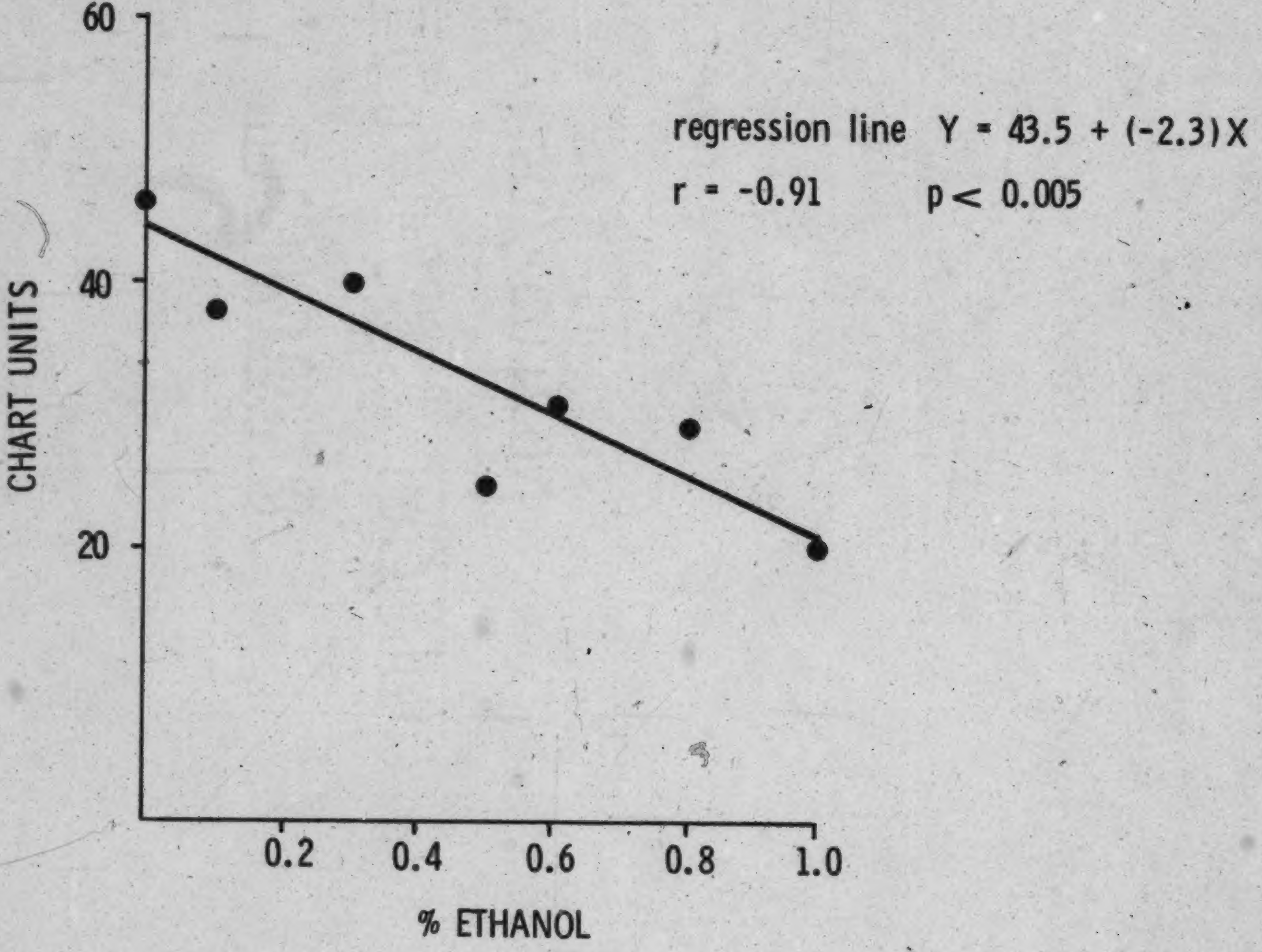


Fig. 28

ADP-INDUCED AGGREGATION: AMPLITUDE
AT THE PEAK OF THE PRIMARY WAVE OF
AGGREGATION vs. ETHANOL CONCENTRATION

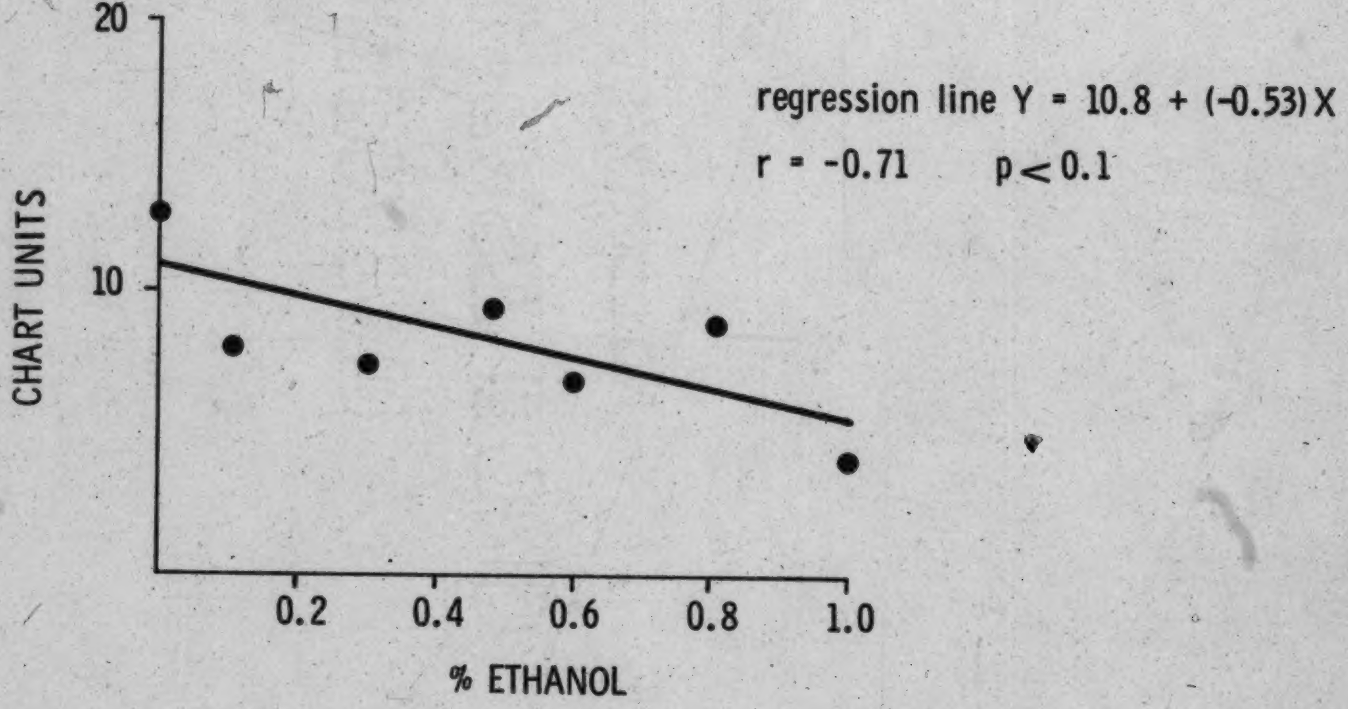
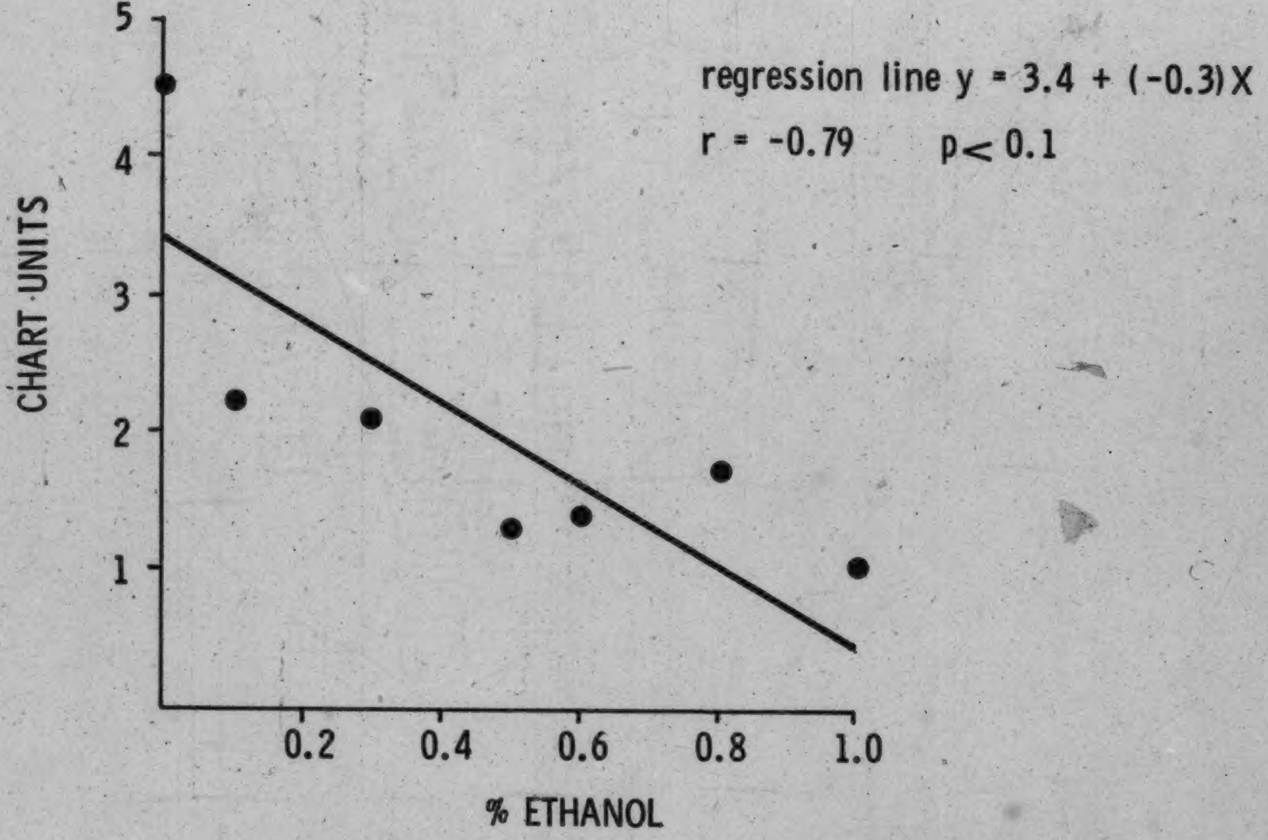


Fig. 29

ADRENALIN-INDUCED AGGREGATION: AMPLITUDE AT THE PEAK OF THE PRIMARY WAVE OF AGGREGATION vs. ETHANOL CONCENTRATION



regression line $y = 3.4 + (-0.3)X$

$r = -0.79$ $p < 0.1$

Fig. 30

ADP-INDUCED AGGREGATION: SLOPE OF THE SECONDARY WAVE OF AGGREGATION vs. ETHANOL CONCENTRATION

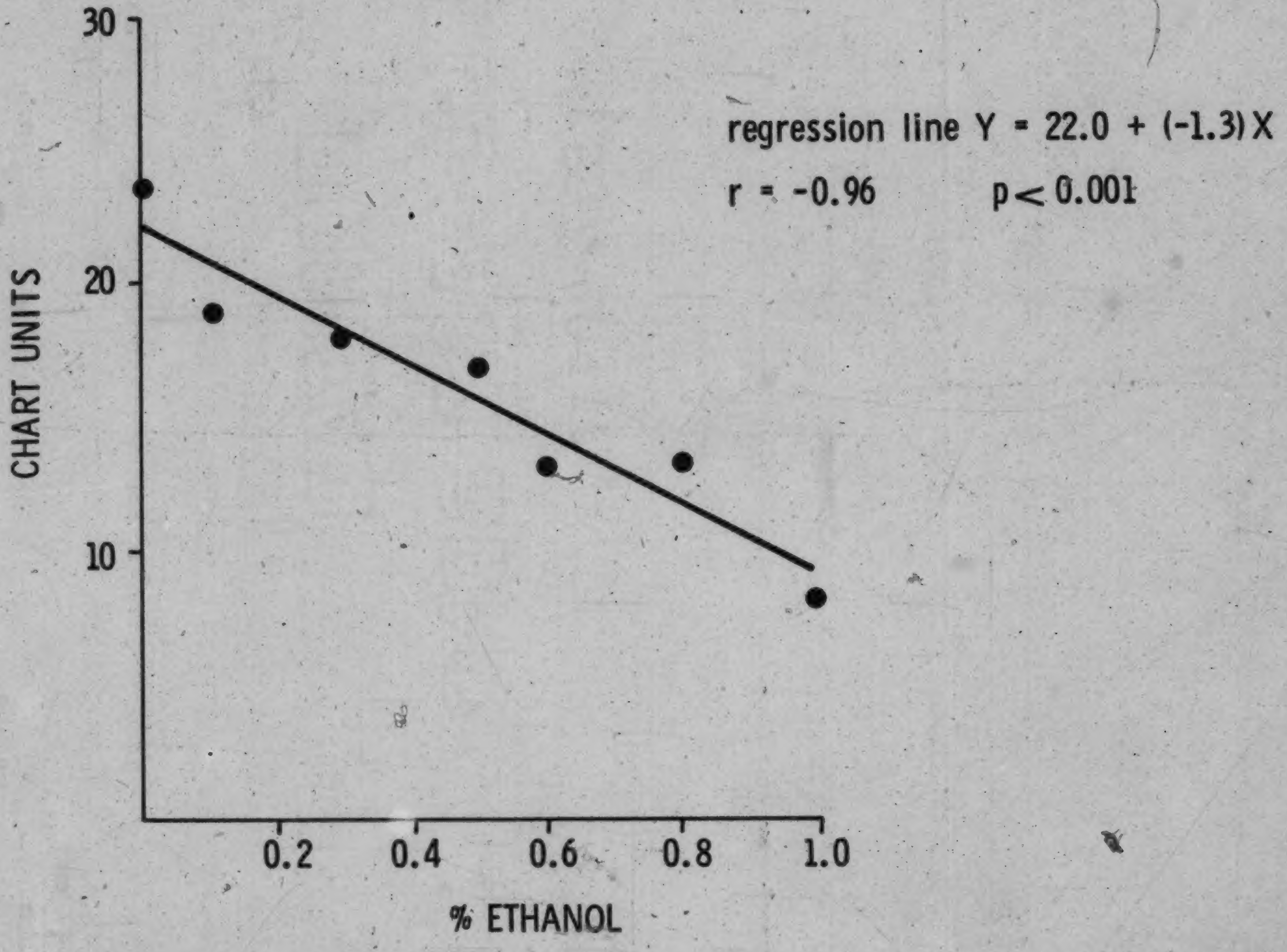


Fig. 31

ADRENALIN-INDUCED AGGREGATION:
SLOPE OF THE SECONDARY WAVE OF
AGGREGATION vs. ETHANOL CONCENTRATION

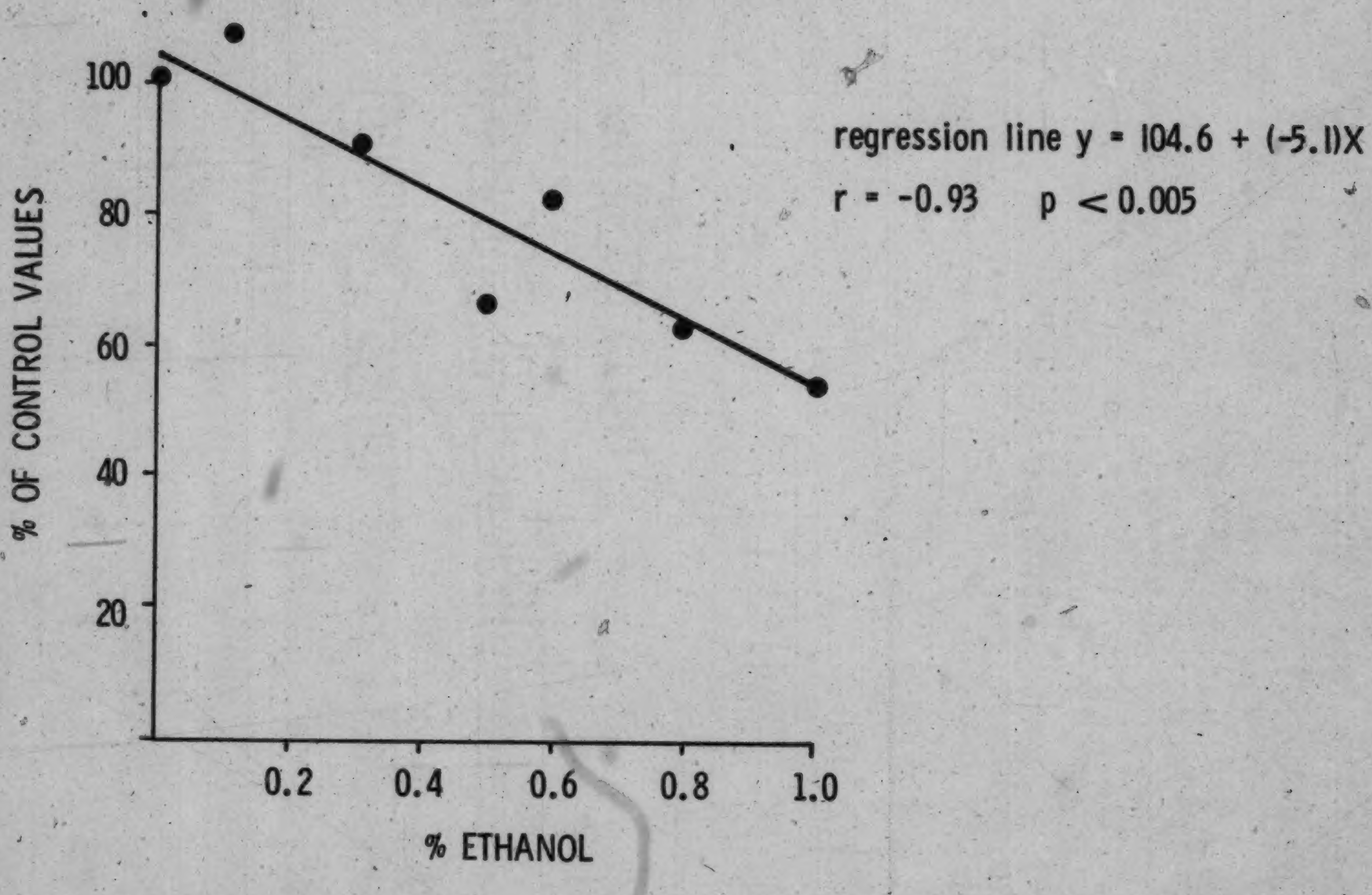


Fig. 32

intrinsic platelet adenosine diphosphate. The defect in platelet aggregation caused by ethanol, on the other hand, affects both the primary and secondary phases of aggregation as evidenced by a decrease in ADP-induced platelet aggregation and changes in the primary wave of epinephrine-induced aggregation. It would seem likely, therefore, that the primary mode of action of ethanol on the platelets is not a disturbance of the release reaction but a more generalized effect such as a change in some aspect of platelet membrane. There are several possible ways in which this could occur.

The first is an effect on platelet lipids, either the lipid bimolecular layer of the surface membrane[15] and/or the phospholipid activity or Platelet Factor 3, which develops late in the release process[82]. Ferrell and Miceli[83] demonstrated a reduction in the incorporation of palmitate-1-^{14}C in total lipids of mouse-liver mitochondrial and microsomal membranes with increasing concentrations of ethanol. These effects occurred primarily in the phospholipid fraction and more specifically in the phosphatidyl ethanolamine and lecithin fractions. They postulated either an increased enzymatic hydrolysis of membrane phospholipids or a decreased synthesis in the presence of alcohol.

A second possible mode of action might be a change in the platelet charge. Platelets are known to carry a net negative charge[84] and must somehow overcome mutually repulsive electrostatic forces before they are able to adhere to one another. Knutsson demonstrated a marked decrease in the membrane potential of frog muscle fibers at concentrations of alcohol equal to and greater than 0.2M [85]. There was a reversal of potential changes after the removal of ethanol. Similar changes in the membrane potential of the platelet might lead to a disturbance of platelet aggregation.

A third mechanism which might be involved in the suppression of aggregation is an alteration of the platelet contractile protein, thrombosthenin. This protein has been recognized for some years as having biochemical and physiological properties similar to those of contractile protein of muscle tissue[86]. However, its exact location within the platelet was unknown.

Recent evidence has been presented for the existence of two distinctly different proteins: (i) the surface localized contractile protein (S-thrombosthenin); and (ii) the cytoplasmic contractile protein (C-thrombosthenin)[88]. Furthermore, a contractile protein model for platelet aggregation has been proposed which involves the formation of intramolecular S-thrombosthenin bridges between adjacent cells, which in turn can contract, drawing the platelets into a tight mass or thrombus[89]. Gimeno et al have recently shown that ethanol at concentrations between 0.1 and 0.88 gm% depresses the contractibility of the rat atria, the relationship between contractile depression and concentration being essentially linear. The effects are reversible in all cases. A simultaneous shortening of the action potential of the atrial cells was also noted and may partly account for the depression of the contractibility but, in addition, there appeared to be some more direct action of the contractile mechanism itself[90]. Considering the physiochemical similarities between thrombosthenin and the contractile protein of muscle tissue, it does not seem unlikely that ethanol might exert a similar effect on platelet thrombosthenin (having a similar linear relationship with concentration) resulting in a decrease in platelet aggregation.

CHAPTER VI

THOUGHTS ON FURTHER STUDY

The present study leaves many questions unanswered. Further work is required to determine the exact cause for the shortened platelet survival in alcoholic patients. There was no evidence of increased liver or splenic sequestration of the platelets and the site and mode of their destruction remains open to speculation.

Morphological and biochemical studies of both the platelets and the megakaryocytes would be interesting to determine the precise effect of alcohol on these cells and the relationship of this change to the ensuing thrombocytopenia and the functional abnormalities evidenced by the disturbance in platelet aggregation. Specifically, electron microscopic studies of normal and "alcoholic" platelets with particular attention to membrane differences would be of interest.

These problems could be argued to be purely academic, as the platelets of alcoholic patients return to normal with no treatment other than the cessation of alcohol. However, increasing numbers of platelet abnormalities are being recognized and the elucidation of the mechanism involved in alcoholic thrombocytopenia might throw some light on the understanding of these other disorders.

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